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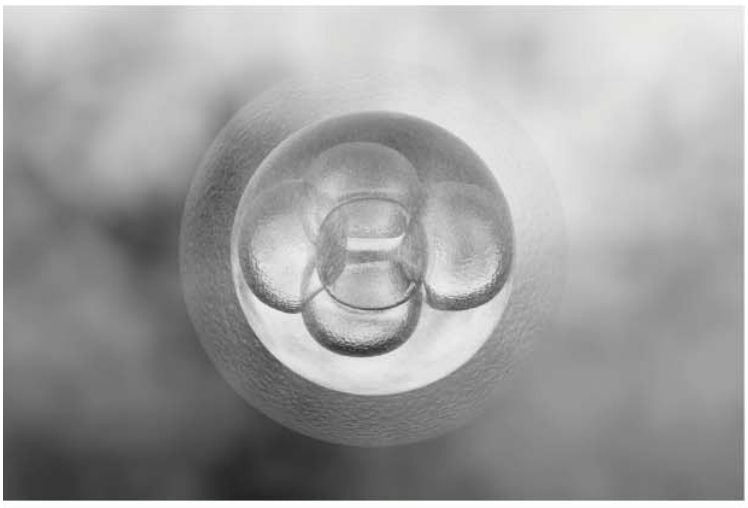

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Abstracts
37th Virtual Annual Meeting of the European Society of Human Reproduction and Embryology

26 June to 1 July 2021

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ORAL

Monday, 28 June 2021

Tuesday, 29 June 2021

(*continued overleaf*)

Wednesday, 30 June 2021

Thursday, 01 July 2021

- **INVITED SESSIONS**
- **SELECTED ORAL COMMUNICATION SESSIONS**
- **POSTER DISCUSSION SESSIONS**

POSTER

Human Reproduction, Vol.36, No.Suppl, pp. i1–i513, 2021

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ESHRE 2021 / Oral presentations

INVITED SESSION

SESSION 01: KEYNOTE SESSION

28 June 2021 Stream 1 08:30 - 09:30

O-001 Delay in IVF treatment up to 180 days does not affect pregnancy outcomes in women with diminished ovarian reserve

P. Romanski[1], P. Bortoletto[1], Z. Rosenwaks[1], G. Schattman[1]

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Abstract text

In clinical practice, infertility treatment delays can occur due to medical, logistical, or financial reasons. Concerns over treatment delays were brought to the forefront in March 2020 when the SARS-CoV-2 pandemic prompted both the ESHRE and ASRM to recommend the suspension of new infertility treatment cycles. At the time, little was known about the risk of viral transmission on reproductive health and necessary medical resources urgently needed to be reallocated to the front lines of the pandemic. These society recommendations were met with resistance from some clinicians and patients that raised valid concerns about whether delaying IVF treatment for a few months could negatively affect pregnancy outcomes.

To help answer this question, we designed a retrospective cohort study to assess whether a delay up to 180 days in initiating IVF treatment affects pregnancy outcomes in infertile women with diminished ovarian reserve. This population was selected because their treatment outcomes were the most likely to affected by treatment delays due to the continuous decline in ovarian reserve over time. Infertile women treated at our IVF center were included if they had diminished ovarian reserve and started an ovarian stimulation cycle within 180 days of their initial consultation that resulted in an oocyte retrieval with planned fresh embryo transfer between 1 January 2012 and 31 December 2018. Diminished ovarian reserve was defined as an anti-Mŭllerian hormone (AMH) <1.1 ng/mL.

In total, 1,790 patients met inclusion criteria (1,115 immediate and 675 delayed treatment). Each patient had one included cycle and no subsequent data from additional frozen embryo transfer cycles were included. Since all cycle outcomes evaluated were from fresh embryo transfers, no genetically tested embryos were included. Patients were grouped by whether their cycle started 1-90 days after presentation (immediate) or 91-180 days (delayed). The primary outcome was live birth (≥24 weeks of gestation). A subgroup analysis of more severe forms of diminished ovarian reserve was performed to evaluate outcomes for patients with an AMH <0.5 and for patients >40 years old with an AMH <1.1 ng/mL (Bologna criteria for diminished ovarian reserve). Logistic regression analysis, adjusted *a priori* for patient age, was used to estimate the odds ratio (OR) with a 95% CI. All pregnancy outcomes were additionally adjusted for the number of embryos transferred.

The mean ± SD number of days from presentation to IVF start was 50.5 ± 21.9 (immediate) and 128.8 ± 25.9 (delayed). After embryo transfer, the live birth rate was similar between groups (immediate: 23.9%; delayed: 25.6%; OR 1.08, 95% CI 0.85-1.38). Additionally, a similar live birth rate was observed in a subgroup analysis of patients with an AMH <0.5 ng/mL (immediate: 18.8%; delayed: 19.1%; OR 0.99, 95% CI 0.65-1.51) and in patients >40 years old with an AMH <1.1 ng/mL (immediate: 12.3%; delayed: 14.7%; OR 1.21, 95% CI 0.77-1.91).

Overall, we observed that a delay in initiating IVF treatment up to 180 days does not affect the live birth rate for women with diminished ovarian reserve when compared to women who initiate IVF treatment within 90 days of presentation. This observation persisted for patients who in the highest-risk categories for poor response to ovarian stimulation. Providers and patients should be reassured that when a short-term treatment delay is deemed necessary for medical, logistical, or financial reasons, treatment outcomes will not be negatively affected.

O-002 Expanding the ovary's reproductive lifespan: Preservation and Rejuvenation

A. Pellicer[1]

[1]IVIRMA- Rome, Reproductive Medicine, Rome, Italy

Study question: The ovary has short lifespan. Genetic and pathologic alterations make it shorter. Moreover, many women delay fertility requiring expanded ovarian function. Can be realistically achieved?

Summary answer: The reproductive lifespan of the ovary can be expanded to a certain extent in physiologic and pathologic (premature ovarian insufficiency (POI)) conditions.

What is known already: In ovaries functioning in physiologic conditions, oocyte cryopreservation (OC) is an established method to expand the reproductive lifespan allowing women to postpone fertility without compromising oocyte's performance. In oncology, ovarian tissue cryopreservation/ ovarian tissue transplantation (OTC/OTT) and OC are widely employed. In POI patients, there are resting follicles in 1/3 of patients. Different techniques have been developed to "awake" these follicles. Some surgical procedures disrupt Hippo signaling to induce primordial follicle growth; , others intend to employ the growth factors contained in blood; some others use bone marrow-derived stem cells to reach similar goals.

Study design, size, duration: A literature search was done to identify the most recent and informative studies on the different techniques applied to increase the reproductive lifespan of the ovaries, including those clinically available, such as OC, and others still considered experimental, such as OCT/OTT, injection of platelets-enriched plasma (PRP), culture-free in vitro activation(CF-IVA), and autologous stem cell ovarian transplantation (ASCOT).

Participants/materials, setting, methods: Outcome of 641 healthy women performing OC and ART cycles. In oncology, OC in 80 women and OTC/OTT in 285 patients willing to conceive was analyzed. Both techniques were compared in the same setting in oncology : 1024 undergoing OC and 800 performing OCT. In POI, we analyzed the outcome of 304 women after PRP; 11 undergoing CF-IVA; and 28 ASCOT patients. The most relevant experimental techniques were also analyzed to understand future directions.

Main results and the role of chance: When it comes to expanding the reproductive function in physiologic conditions, mostly due to delay in childbearing, the follow-up of 641 women out of 1073 who underwent OC and subsequent embryo transfer (ET) has shown 68.8% cumulative live birth rates (C-LBR). Age matters because C-LBR decreased >50% after age 35 yrs. If only the endocrine function of the ovary is considered, OCT/OTT has consistently shown almost 86% efficacy.

In Oncology, OC provided 42.1% C-LBR in 80 individuals after cure, while the follow-up of 285 women from 5 different centers after OCT/OTT yield 26% LBR. Both OC and OTT were compared in the same setting and OC proved to be slightly better, with 32.6% LBR as compared to 22.8% in OCT/OTT.

Regarding POI, the use of intraovarian PRP injection in 304 women displayed 8% LBR; CF-IVA 36.3% LBR in 11 women; and ASCOT 10% LBR in 10 POI patients and 27.8% in 18 poor responders (PR).

Experimental data suggest that a combination of ASCOT and PRP must be the best alternative to activate dormant follicles in POI women.

Limitations, reasons for caution: None of the studies was a RCT, and many had not controls, most are descriptive. Regarding oncology patients OC is save and reassuring. The experience shows that OCT/OTT is also safe, although some Scientific Societies label OCT/OTT still as experimental. All the techniques employed in POI are experimental yet.

Wider implications of the findings: Expanding the reproductive lifespan of the ovary in health and disease (oncology and others) employing OC is a routine; OCT/OTT can be also applied to expand the endocrine function of the ovaries. The best and less invasive method to activate follicles in POI and PR still needs to be defined.

Trial registration number: NCT02240342; NCT03535480; NCT04475744; NCT02354963

SELECTED ORAL COMMUNICATIONS

SESSION 02: SAFETY FOLLOW-UP ON ART CHILDREN; DATA FROM INFANT TO YOUNG ADULT

28 June 2021 Stream 1 10:00 - 11:30

O-072 Markers of cardiometabolic health of adolescents conceived through assisted reproductive technologies (ART) appear reassuring

L. Wijs[1], D. Doherty[2], J. Keelan[1], P. Burton[3], J. Yovich[4], L. Beilin[5], T. Mori[5], R.C. Huang[6], L. Adams[5], J. Olynyk[7], O. Ayonrinde[8], R. Hart[9]

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Study question: Is the cardiometabolic health of adolescents conceived through ART worse than that of their spontaneously-conceived counterparts?

Summary answer: The majority of cardiometabolic and vascular health parameters of ART-conceived adolescents are more favourable than those of their spontaneously-conceived counterparts of similar age.

What is known already: It has been proposed that ART induces epigenetic alterations during embryonic development which could lead to cardiometabolic disease later in life. However, individuals requiring ART may themselves be metabolically less healthy than the general population, which could lead to a genetically increased risk of cardiometabolic disorders in the offspring, rather than the ART procedure. The literature pertaining to cardiometabolic health of ART-conceived offspring is contradictory, but generally suggests unfavourable cardiometabolic health parameters. With over 8 million children and adults born through ART worldwide, it is imperative to investigate whether early signs of adverse cardiometabolic differences persist into adolescence and beyond.

Study design, size, duration: The Growing Up Healthy Study (GUHS) is a prospective study that recruited 303 ART-conceived adolescents, born 1991-2001 in Western Australia. Their health parameters, including cardiometabolic factors, were assessed and compared with spontaneously conceived counterparts of similar socioeconomic background and age from the Raine Study Generation 2 (Gen2). The 2868 Gen2 participants were born 1989-1992 and are representative of the Western Australian adolescent population. At age 16-17 (2013-2017), GUHS participants replicated assessments previously completed by Gen2.

Participants/materials, setting, methods: Cardiometabolic parameters were compared between 165 GUHS (male=50.9%) and 1690 Gen2 (male=49.8%) adolescents. Assessments consisted of a detailed questionnaire; health and demographic parameters, anthropometric assessments; height, weight, body-mass index (BMI), waist circumference and skinfold thickness, fasting serum biochemistry, arterial stiffness and blood pressure assessment using applanation tonometry, assessment of non-alcoholic fatty liver (NAFLD) and thickness of abdominal fat compartments using ultrasonography. Chi^2, Fisher's Exact and Mann-Whitney U tests, performed in SPSS V25, examined cohort differences.

Main results and the role of chance: GUHS adolescents appeared to be healthier from a cardiometabolic perspective than their Gen2 counterparts. They were leaner, with lower BMI (median: 21.23 vs. 22.06, P=0.004), lower waist circumference (median: 74.10 vs. 76.75 cm, P=0.031), and thinner skinfolds (triceps median: 12.1 vs. 14.0 mm, P=0.019, subscapular median: 10.6 vs. 11.9 mm, P<.001, mid-abdominal median: 16.0 vs. 19.9 mm, P<0.001, supraspinal median: 10.7 vs. 13.5 mm, P<0.001). No significant differences were detected in the following serum fasting parameters: glucose, insulin, HOMA-IR, LDL cholesterol, total cholesterol, cholesterol/HDL-ratio, triglycerides, CRP and ALT. HDL cholesterol levels were more favourable in GUHS (P<0.001). NAFLD was present in 10.9% of GUHS vs. 15.2% of Gen2 adolescents (P=0.174), with no difference in steatosis severity score (P=0.309). ART offspring had less subcutaneous adipose tissue (median: 8.0 vs. 14.0 mm, P<.001), more visceral adipose tissue (median: 40.0 vs. 32.0 mm, P<0.001), with no difference in pre-peritoneal adipose tissue (P=0.087). Measures of arterial stiffness were lower in GUHS. Pulse wave velocity: median 6.1 vs. 6.4 m/s, P<0.001 and heart rate corrected augmentation index: median -10.25 vs. -8.00, P=0.006. No significant differences in blood pressure or heart rate were detected. Stratification by sex did not greatly alter the results.

Limitations, reasons for caution: Despite the substantial study size and the unique study design, we were unable to differentiate between different types of ART (e.g. IVF vs. ICSI), draw definite conclusions or relate outcomes to cause of infertility. Given the observational character of this study, causation cannot be proven.

Wider implications of the findings: In this study we did not detect any adverse effect of ART on cardiometabolic health at adolescence, in contrast to some studies. Given the lack of consensus, future well-designed and appropriately-powered studies are necessary to investigate cardiometabolic health in ART adults.

Trial registration number: not applicable

O-073 Perinatal outcomes of infants conceived using partner versus donor sperm - An analysis of singleton and twin pregnancies from the UK national dataset

C. Allen[1], D. McLernon[1], S. Bhattacharya[1], A. Maheshwari[1]

[1]University of Aberdeen, Applied Health Sciences, Aberdeen, United Kingdom

Study question: Are perinatal outcomes different in pregnancies conceived using donor sperm compared with those with partner sperm?

Summary answer: The perinatal outcomes of singleton and twin pregnancies conceived with donor sperm are better when compared to those conceived with partner sperm

What is known already: There has been a substantial increase in the use of donor sperm in the last 15 years across the world. A recent systematic review and meta-analysis has suggested that there is an increased risk of hypertensive disorders of pregnancy and small for gestational age babies from ART treatment using donor sperm compared to partner`s sperm. This meta-analysis was limited due to poor quality of primary studies often with small sample sizes.

Study design, size, duration: This is a retrospective cohort study on 196,293 singleton and 46,275 twin pregnancies from the Human Fertilisation

and Embryology Authority (HFEA) anonymised dataset including all live births from 1991 to 2016. Outcomes were preterm birth (< 37 weeks); very preterm birth (<32 weeks); very low, low, high and very high birth weight (<1500g, <2500g, >4000g and >4500g respectively); congenital anomaly and healthy baby (term live birth with appropriate weight and no congenital anomaly).

Participants/materials, setting, methods: All pregnancies resulting in singleton or twin livebirth were included. Any cycle involving donor oocytes, PGD, gamete intra-fallopian transfer, ectopic pregnancy, miscarriage, stillbirth, or termination was excluded. Logistic regression and generalised estimating equations were used for analysis of singletons and twins, respectively. Odds ratios (aOR) with 95% confidence intervals (CI) for donor versus partner sperm were adjusted for maternal age, previous pregnancy, cause of infertility and year for all outcomes plus gestational age for birthweight.

Main results and the role of chance: Baseline characteristics for donor and partner sperm pregnancies were assessed for singleton and twin livebirths separately. In both analyses there were significant differences between donor and partner sperm pregnancies in terms of maternal age, previous pregnancy status and cause of infertility.

Analysis of singleton births demonstrated an increased odds (aOR, 95% CI) of having a healthy baby (1.09, 1.05 - 1.12) and reduced odds of congenital anomaly (0.34, 0.29 - 0.39), very preterm birth (0.66, 0.58-0.75), preterm birth (0.81, 0.76-0.86), low birthweight 0.89 (0.83 - 0.96) in singleton births using donor sperm compared with those using partner sperm. There was, however, an increased odds of high birthweight (1.10, 1.05 - 1.16) and very high birthweight (1.16, 1.05-1.29) with donor sperm pregnancies.

Analysis of twin births conceived with donor sperm also showed higher odds of having a healthy baby (1.07, 1.01 - 1.15) and lower odds of congenital anomaly (0.52, 0.39 - 0.68) compared with partner sperm. There were no statistically significant differences between the birthweight or birth gestation outcomes for twin pregnancies.

Sensitivity analysis of only cases with complete outcome data showed no significant differences when compared to the primary analysis.

Limitations, reasons for caution: This is a retrospective study of a single nation's routinely collected data. We could not adjust for confounders such as smoking, BMI and pregnancy complications such as pre-eclampsia, as they are not recorded in HFEA's dataset.

Wider implications of the findings: Patients and clinicians can be reassured that donor sperm pregnancies are not at higher risk of adverse perinatal outcomes. In fact, they are more likely to result in a healthy baby. Worldwide registries should consider including maternal data to enable a better assessment of outcomes.

Trial registration number: Not applicable

O-074 No methylome differences observed in IVF children born after embryo culture in different culture media

R. Koeck[1,2], J. Tost[3], F. Busato[3], D. Consten[4], J. Van Echten-Arends[5], S. Mastenbroek[6], Y. Wurth[4], H. Zandstra[7], R. Van Golde[7], J. Dumoulin[7], H. Brunner[2,8], M. Zamani Esteki[1,2,9], A. Van Montfoort[7,9]

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*[9]*joint last author, x, The Netherlands*

Study question: Does human embryo culture in different IVF culture media lead to DNA methylation alterations in IVF offspring?

Summary answer: Genome-wide analyses identified no significant DNA methylation differences between culture medium groups in IVF children (neonates or 9-year olds) from two culture media studies.

What is known already: During in vitro fertilisation (IVF) treatments, embryos undergo preimplantation development in an artificial environment, while concurrently undergoing epigenetic reprogramming. Adversity during this period, such as peri-conception calorie restriction, has been linked to persistent DNA methylation aberrations and increased risk of cardiometabolic disease. Early environmental adversity is suspected in IVF offspring as they are born with lower birthweights and show increased risk of cardiometabolic dysfunction in adulthood as compared to their naturally-conceived counterparts. This is further supported by the observation from two culture media trials (MEDIUM0 and MEDIUM1) that embryo culture in different culture media leads to differences in birthweight.

Study design, size, duration: We recruited singleton offspring from two IVF culture media trials. The MEDIUM0 study, a pseudo-randomized trial comparing G3 (Vitrolife) and K-SICM (Cook), was conducted from 2003-2006. At the 9-year follow-up, saliva was collected (cohort-A). The MEDIUM1 study, a multi-center randomized controlled trial comparing G5 (Vitrolife) and HTF (Lonza), was conducted from 2010-2012. Umbilical cord blood (UCB) was collected at birth (cohort-B).

Participants/materials, setting, methods: DNA methylation was analysed in 120 saliva samples (65 G3, 55 Cook) and 106 UCB samples (47 HTF, 59 G5) using the Infinium MethylationEPIC array (Illumina). Mixed effects linear models, correcting for (gestational) age, sex, sample composition and batch effects alongside maternal age, pregnancy complications and IVF centre for cohort-B, were implemented at single or aggregated sites. Methylation outliers were defined as values over three interquartile ranges below or above 25th and 75th percentiles respectively.

Main results and the role of chance: 111 of the 120 saliva samples (60 G3, 51 Cook) and 105 of the 106 UCB samples (47 HTF, 58 G5) passed our quality control criteria. We filtered sites on sex chromosomes, and based on quality, proximity to single-nucleotide polymorphisms, and proportion of missing values, leaving 650,000-700,000 of the 850,000 sites included on the EPIC array for our analyses. To account for heterogeneity in the cellular composition of our samples we estimated their cell compositions using a reference-based approach. First, we investigated individual CpG sites, finding no differentially methylated sites in either cohort after correction for multiple testing (false discovery rate adjusted p. value threshold <0.1). Sites were then aggregated into regions based on their allocations to genes, promoters and CpG islands. No differentially methylated regions were identified in either cohort. A targeted analysis of DNA methylation of imprinting genes showed no differentially methylated sites or regions. To examine the contribution of stochastic epigenetic alterations we quantified the number of methylation outliers per sample. Although this revealed a predominance of hypomethylation outliers, there was no difference in the total number or distribution of DNA methylation outliers between the two culture media groups of cohort-A and cohort-B.

Limitations, reasons for caution: This analysis is currently limited by the lack of comparison to a naturally-conceived control group. As such, we cannot yet conclude whether IVF embryo culture, in any medium, is associated with DNA methylation aberrations. Additionally, given the large number of comparisons, we may lack power to detect small differences.

Wider implications of the findings: Although there are disparities in birth weight and childhood growth after embryo culture in different media, we observed no DNA methylation alterations preserved postnatally. Whether DNA methylation of these individuals deviates from that of naturally-conceived individuals will be determined in the near future.

Trial registration number: MEDIUM1: NTR 1979 /NL1866 (Netherlands Trial Registry)

O-075 The association between high birth weight and long-term outcomes-implications for Assisted Reproductive Technologies: a systematic review and meta-analysis

Å. Magnusson[1], H. Laivouri[2], A. Loft[3], N. Oldereid[4], A. Pinborg[3], L.B. Romundstad[5], M. Petzold[6], V. Söderström-Anttila[7], C. Bergh[1]

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Study question: Do high birth weight or large for gestational age (LGS) increase the risk of serious disease later in life?
Summary answer: High birth weight and/or LGA were associated with elevated risks for certain child malignancies, breast cancer, psychiatric disorders, childhood hypertension and diabetes type 1.
What is known already: Previous studies have shown that children born after frozen embryo transfer (FET) have an increased risk of being born LGA or having a high birth weight. In recent years the practice of FET in Assisted Reproductive Technology (ART) has increased rapidly. The perinatal risks of being born LGA or with a high birth weight are well studied, however less is known about the impact on long-term health and morbidity.
Study design, size, duration: Pubmed, Scopus and Web of Science were searched until December 2020. 11 748 abstracts were screened, 172 publications were selected for systematic review and 63 for meta-analyses. The methodological quality in terms of risk of bias was assessed by pairs of reviewers. Robin-I (www.methods.cochrane.org) was used for assessing risk of bias in original articles. For systematic reviews AMSTAR was used. For certainty of evidence the GRADE system was used.
Participants/materials, setting, methods: Exposures were LGA and high birth weight. Long-term morbidity outcomes were cancer, metabolic disease, cardiovascular disease and psychiatric disorders. Cancer was focused on breast cancer, child malignancies in the central nervous system (CNS), hematological malignancies and Wilm´s tumor. Metabolic diseases included diabetes type 1 and type 2. Cardiovascular diseases were focused on hypertension and other cardiovascular disorders and psychiatric disorders on schizophrenia/psychosis and cognitive disorders.
Main results and the role of chance: Pooled Adjusted Odds Ratios (AOR) for outcome variables were compared for birth weights >4000 or >4500 g versus <4000 g. For cancer, meta-analyses showed AOR of 1.24 (95% 1.11-1.39) for development of breast cancer, AOR of 1.15 (95% CI 1.05-1.27) for development of CNS tumors, AOR of 1.29 (95% CI 1.20-1.39) for childhood leukemia and AOR 1.68 (95% CI 1.38-2.06) for Wilm´s tumor.

For metabolic disease a meta-analysis showed AOR of 1.15 (95%CI 1.05-1.26) for the association between high birth weight and type 1 diabetes.

For psychiatric diseases an association was found between high birth weight and/or LGA and schizophrenia and depression.

For cardiovascular disease, an association was found between high birth weight and hypertension in childhood with an inverse association in adulthood. No difference in the risk of coronary heart disease in adults born with high birth weight compared to normal birth

Limitations, reasons for caution: The main limitation is that all data are based on observational studies with their inborn risk of selection bias. Our conclusions are however, mainly based on meta-analyses and/or studies with low risk of bias.
Wider implications of the findings: Even though high birth weight and LGA are associated with an increased risk of serious diseases, both in childhood and in adulthood, the size of these effects seems modest. However, the identified risk associations should be taken into account in stimulation strategies and when considering fresh or frozen embryo transfer.
Trial registration number: Not applicable

O-076 Neurodevelopmental morbidity in children born after ART: a Nordic register study from the Committee of Nordic ART and Safety (CoNARTaS) group

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Study question: Does the risk of neurodevelopmental disorders differ between singletons born after various assisted reproductive techniques (ART) and spontaneous conception (SC) until young adulthood?
Summary answer: ART children had a slightly increased rate of learning and motor functioning disorders, autism spectrum disorders (ASD), and ADHD and conduct disorders.
What is known already: Studies on the impact of ART on offspring have reported both increased risk and comparable incidences of neurodevelopmental disorders between ART and SC offspring. The most studied neurodevelopmental disorders with ART are autism spectrum disorders (ASD.) There is, however, no consensus on the risk of ASD for ART children. The risk for other neurodevelopmental disorders, like attention-deficit hyperactivity disorders (ADHD) or tic disorder among ART children, is also a debated issue, as studies are scarce.
Study design, size, duration: A Nordic register-based cohort study including all singleton live births (N = 5 076 444) after ART (n= 116 909) or SC (n = 4 959 535) between 1995 and 2014 in Denmark and Finland, 1995 and 2015 in Sweden; and 2005 and 2015 in Norway. Children with intellectual disability (ICD-10: F70-F79) are excluded. The children are followed up to young adulthood (the year 2014 in Denmark and Finland, and 2015 in Norway and Sweden).
Participants/materials, setting, methods: Offspring outcomes were defined as following ICD-10 diagnoses: learning and motor functioning disorders (F80-83), ASD (F84), ADHD and conduct disorders (F90-F92), and tic disorders/Tourette (F95). We calculated crude and adjusted hazard ratios (HR) for neurodevelopmental diagnoses using Cox regression. Adjustments were made for the country, maternal age at the delivery, parity, smoking, and maternal psychiatric morbidity.
Main results and the role of chance: The cumulative incidences of neurodevelopmental disorders in the cohort were 1.74% for F90-F92, 1.40% for F80-83, 0.66% for F84, and 0.22% for F95. In crude Cox-regression ART children had an increased likelihood during the follow-up of being diagnosed with F84 (HR 1.12 [95% CI 1.04-1.21]) and F95 (HR 1.21 [95% CI 1.06-1.38]), but not with F80-83 (HR 1.01 [95% CI 0.96-1.07]) or F90-92 (HR 0.82 [95% CI 0.77-0.86]). After adjustments the likelihood was increased for F80-83 (HR 1.20 [95% CI 1.13-1.27]), F84 (HR 1.12 [95% CI 1.03-1.24]), and F90-92 (HR 1.09 [95% CI 1.04-1.19]), but nor for F95 (HR 1.13 [95% CI 0.99-1.30]).

After adjustments, intracytoplasmic sperm injection children compared with in vitro fertilization children had similar likelihood during follow-up for F80-83 (1.06 [95% CI 0.89–1.25]), for F84 (HR 0.92 [95% CI 0.76–1.11]), for F90-92 (HR 0.96 [95% CI 0.83–1.12]), and for F95 (HR 1.16 [95% CI 0.83–1.63]).

After adjustments, frozen embryo transfer children compared with fresh embryo transfer children had similar likelihood during follow-up for F80-83 (HR 1.11 [95% CI 0.90–1.37]), F84 (HR 0.98 [95% CI 0.76–1.27]), F90-92 (HR 0.96 [95% CI 0.78–1.19]), and F95 (HR 0.83 [95% CI 0.51–1.35]).
Limitations, reasons for caution: There may be residual confounding by unknown or unmeasured confounders. We lack information on possible confounders like the reason and length of infertility, maternal substance use other than self-reported smoking status, paternal age, and parental somatic morbidity. Additional limitations are differences in registration practice and data availability between study countries.
Wider implications of the findings: This is the largest singleton cohort and the first multinational study on the risk for neurodevelopmental disorders among ART children. While the rate of some neurodevelopmental disorders was increased among ART children, the absolute risk was moderate. The type of ART did not associate with the incidence of neurodevelopmental disorders.
Trial registration number: ISRCTN11780826

O-077 Cancer risk in a nationwide cohort of children and young adults conceived by assisted reproductive technology in 1983-2012

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[5]Amsterdam UMC- Vrije Universiteit Amsterdam, Department of Obstetrics & Gynecology- Amsterdam Reproduction & Development research institute, Amsterdam, The Netherlands

Study question: Are children conceived by assisted reproductive technology (ART) at increased cancer risk, compared with the general population and with non-ART conceived offspring from subfertile women?
Summary answer: Overall cancer risk was not increased in ART-conceived offspring compared with non-ART conceived offspring from subfertile women (median follow-up, 17 years).
What is known already: There is growing evidence that ART procedures could perturb epigenetic processes during the pre-implantation period. Although the results of most studies are reassuring for children born after in vitro fertilization (IVF), recent studies showed (non-)significantly increased cancer risks after intracytoplasmic sperm injection (ICSI) and frozen embryo transfer (FET). Since the proportion of children born after these techniques increased dramatically over the past decades, it is important from a public health perspective to investigate cancer risk after ICSI and FET in larger studies.
Study design, size, duration: Data were used from the OMEGA-cohort, a historical nationwide cohort with prospective follow-up in the Netherlands. Offspring of women who were treated in one of the 13 IVF clinics or 2 regional fertility centers between 1983-2012 were included. Of 98,165 live-born children, 53,154 were ART-conceived and 45,211 were non-ART conceived (conceived naturally with or without ovarian hyperstimulation) by subfertile women.
Participants/materials, setting, methods: Data on type of fertility treatment and maternal risk factors were available from medical records from the mothers and the Dutch Perinatal registry. Cancer incidence was ascertained through linkage with the Netherlands Cancer Registry. Cancer risk in ART-conceived children was compared with risk in children not conceived by ART from subfertile women (hazard ratios [HRs]) and with children from the general population (standardized incidence ratios [SIRs]).
Main results and the role of chance: The median age at end of follow-up was 17 years and was shorter in ART-conceived children (16.1 years) compared with non-ART children (19.1 years). In total, 382 cancers were observed, 166 in the ART group and 222 in the non-ART group. In preliminary analyses, overall cancer risk was not increased in ART-conceived children, neither compared with children not conceived by ART from subfertile women (HR:0.98, 95% confidence interval (CI)=0.79-1.22) nor compared with the general population (SIR:0.98, 95% CI=0.81-1.11). Risks were also not significantly increased in children conceived by ICSI or FET (HR:1.20, 95%CI=0.85-1.70; 1.25, 95%CI=0.68-2.43, respectively). From 18 years of age onwards, the HR of cancer in ART-conceived versus non-ART individuals was 1.22 (95%CI=0.86-1.74). There were no significantly increased site-specific cancer risks in ART-conceived children compared with non-ART children and the general population. Risk of lymphoblastic leukaemia was not increased in the ART group compared with the non-ART group (HR: 1.03, 95% CI=0.58-1.82).
Limitations, reasons for caution: Despite the large cohort and long-term follow-up the number of cancer cases was limited which hampered some subgroup analyses, especially for analyses according to specific cancer types and children born after FET.
Wider implications of the findings: The results from this study importantly contribute to the current knowledge about health risks in ART-offspring. Physicians may inform parents who consider ART about potential health risks for ART-conceived children. Furthermore, pediatric oncologists caring for ART-conceived children/adolescents with cancer need evidence-based information about the association between ART and cancer risk.
Trial registration number: n.a.

SELECTED ORAL COMMUNICATIONS

SESSION 03: MOLECULAR ADVANCES IN REPRODUCTIVE ENDOCRINOLOGY

28 June 2021 Stream 2 10:00 - 11:15

O-078 Predictive factors of autologous Oocyte Post-warming Survival rate

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Study question: Are there any clinical or paraclinical predictive factors of Oocyte Post-warming Survival (OPS) rate?
Summary answer: Woman age, Body mass Index, estradiol level on triggering day and estradiol/oocyte ratio are critical predicting factors that should be considered before performing oocyte vitrification.
What is known already: Since the development and the validation of oocyte vitrification, we vitrify oocytes in different medical situations for patients who benefit ICSI. Although the OPS rate in our centre is satisfying, occasionally, it happens to be lower. OPS is dependent on quality of oocyte as demonstrated by the difference of OPS in oocyte donation/autologous cycles. The present study questions the existence of clinical and paraclinical factors predicting in OPS. In order to tackle this issue, we have assessed several parameters related to the woman and to her response to hormonal treatment known to influence oocyte quality in relation to OPS
Study design, size, duration: A retrospective observational study of 786 autologous oocyte vitrification cycles was performed from October 2011 to July 2018 in 5 situations: cycles where only a part of mature collected oocytes were vitrified [1] Partial oocyte vitrification program(n=605), [2] Patients opposed to embryo cryopreservation(n=2) and oocyte freeze-all cycles for the following reasons [3] Uncontrolled Ovarian hyperstimulation(=89), [4] Unfavorable uterine environment/receptivity(n=71) and [5] Absence of spermatozoa(n=20). 1175 warming cycles were analyzed to identify predictive factors for OPS.
Participants/materials, setting, methods: Oocytes were vitrified/warmed using Kitazato media and system. The ratio of OPS survival was measured between the number of intact oocytes and the number of warmed oocytes. The factors assessed as potential predictors of OPS were: woman age, body mass Index (BMI), Estradiol level on triggering day (E2), E2/ number of recovered oocytes (EOR), number of recovered oocytes and maturity ratio (number of mature oocytes/ number of recovered oocytes). Statistics were performed using SPSS software.
Main results and the role of chance: A total of 1175 studied warming cycles were performed and 5421 oocytes were warmed with a mean OPS rate of 84,6% (±22,6). OPS rates were comparable in all situations: [1] 3084/3688 (83,6%), [2] 6/6 (100%), [3] 931/1121 (83,1%), [4] 393/458 (85,8%), [5] 125/148 (84,5%). The mean woman age (33,2 years±4,9 *vs* 33,1 years ±4,3), mean woman BMI (23,1 kg/m2±3,9 *vs* 22,9 kg/m2±4,2), mean E2 (2587,7pg/ml±1140,5 *vs* 2513,2pg/ml±1098,7), mean EOR (207,5pg/ml±119,4 *vs* 196,0pg/ml ±119,4), mean number of total recovered oocytes (15,0±6,8 *vs* 14,7±6,8), mean maturity ratio (85,4%±13,7 *vs* 86,0%±14,2) showed no statistical difference in women with reduced OPS (≤85%) as compared to women with standard OPS (>85%). Subgroups analyses revealed significant higher occurrence of reduced OPS in advanced age women (>40years) (OR=2,4; [95%CI:1,3-4,4] p<0,05) as compared to women of other age categories: <30years (OR=0,5; [95%CI:0,2-0,9]), 30-35years (OR=0,4; [95%CI:0,2-0,7]), 36-40years

(OR=0,2; [95%CI: 0,3-0,5]). The combination of advanced age with abnormal BMI (<18,5 or >24,9kg/m2: OR=7,3[95%CI:1,6-34,0] p<0,01), or elevated E2 (>3000pg/ml: OR=3,3[95%CI:1,0-11,0] p<0,05) or atypical EOR (<140 or >250pg/ml: OR=3,7[1,1-12,2] p<0,05) amplified the risk of reduced OPS. Women with abnormal BMI combined with elevated E2 (OR=2,1[95%CI:1,1-3,9] p<0,05) or atypical EOR (OR=1,6[95%CI:1,0-2.6] p<0,05) were also at higher risk of reduced OPS.

Limitations, reasons for caution: Oocyte vitrification is a manual technique that depends on the skill of the operator. Inter-operator variability was not taken into account in our statistical analyses neither were data regarding ovarian stimulation protocols nor were infertility etiologies.

Wider implications of the findings: This work enabled to identify patient or treatment related factors that highly influence the outcome of oocyte vitrification/warming cycles. Our findings will likely help refining criteria for the selection of candidate patients for oocyte vitrification or to cancel bad prognosis cycles.

Trial registration number: NA

O-079 Could ovarian reserve be affected after SARS-CoV-2 infection?

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Study question: Is there a variation in ovarian reserve in women who have passed the disease?

Summary answer: The fact of having passed SARS-CoV-2 does not affect the ovarian reserve status

What is known already: Despite the overwhelming magnitude of this pandemic and its worldwide prevalence, information regarding the effects of the novel coronavirus on human reproduction are currently limited. As the assisted reproductive technology programs resumed operations, it was important to gather information regarding the status of individuals infected with the novel coronavirus, and to assess gametes and reproductive outcomes for those who had SARS-CoV-2 virus. Since it was described the presence of receptors of the virus in the ovary, studies on the reproductive involvement of coronavirus infection are warranted, particularly within recovered patients

Study design, size, duration: During the period May-June 2020, women performing an Assisted Reproductive treatment in any of the 11 clinics belonging to the IVIRMA group in Spain and who had a positive IgG for SARS-CoV-2 were invited to participate in the study; this group of women had a previous AMH determination of no more than 6 months. The study was approved by an Institutional Review Board (2007-MADR-052-AR) and all women provided written informed consent.

Participants/materials, setting, methods: A new AMH determination was made (Elecsys® AMH, Roche Diagnostics) to detect possible variations in the hormone levels. Women were stratified in two groups, according their previous AMH levels: low responders (AMH<1 ng/ml) or normo-high responders (AMH ≥ 1 ng/ml) Statistical analyses were performed using the Statistical Package for Social Sciences 19.0 (IBM Corporation, Armonk, NY, USA).

Main results and the role of chance: After filtering by the inclusion criteria described above, we included 46 patients in this phase of the study; 16 women were diagnosed as having low ovarian reserve (AMH < 1 ng/ml), with an average age of 38.6 years, whereas 30 were classified as having normal ovarian reserve (AMH ≥ 1 ng/ml), with an average age of 34.7 years. Generally, the data show no variation in AMH levels before and after SARS-CoV-2 infection (1.73 ng/ml vs. 1.61 ng/ml, respectively). However, when we analyzed these differences according to the study groups, we observed that, in women with normal ovarian reserve, average AMH level before infection was 4.6 ng/ml, whereas after infection AMH decreased to 3.1 ng/ml. For women with low ovarian reserve, AMH was 0.8 ng/ml before infection and remained at a similar value after infection (AMH=0.7 ng/ml).

Limitations, reasons for caution: This is an observational study and thus possible confounders cannot be completely excluded. More data are needed to draw firm conclusions it will be critical to increase the sample size to check if the results observed in this work remains in the general population

Wider implications of the findings: The fact of having passed the disease does not affect the ovarian reserve status but the degree of the variation of AMH levels depending on the patient were low or high responder. Nevertheless, we could assume that the chances of success of the Assisted Reproductive treatment remain intact.

Trial registration number: Not apply

O-080 Activated AKT/mTOR signalling in peripheral blood of women with premature ovarian insufficiency and its correlation with variable FMR1 expression profiles

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Study question: How predictive are gene expression levels of AKT/mTOR-signalling-pathway genes in peripheral blood of patients with premature ovary insufficiency (POI) and is there a link to *FMR1*-expression?

Summary answer: *AKT1, TSC2, mTOR, S6K* and *FOXO3*-expression-levels are significantly upregulated in POI-patients and demonstrate a positive correlation with *FMR1*-expression-level in case of *mTOR-, S6K* and *FOXO3*.

What is known already: The AKT/mTOR-signalling-pathway is involved in a range of cellular functions. In female germline it regulates early follicular-activation and follicular-pool-maintenance. Over the past few years AKT-activation has been experimentally applied to induce follicular maturation in POI-patients. Additionally, first evidence of a linked *FMR1* – AKT/mTOR signaling in female germline have been reported.

FMR1 is a major control gene in folliculogenesis. Due to increased (CGG)-triplet-numbers (54<n<200) in its 5'-untranslated-region, named premutation, increased *FMR1*-expression-levels and reduced FMRP-production have been described, associated with POI in 20% of cases. A former study found premutation independent, large transcript-level-variances of *FMR1* in leukocyte RNA-samples of POI-patients.

Study design, size, duration: 74 POI patients and 56 fertile controls were prospectively enrolled in this study. Accordingly, expression levels of genes associated with the AKT/mTOR-signaling pathway and *FMR1* were analyzed and correlated on the mRNA level of their leukocytes.

Participants/materials, setting, methods: All patients provided written informed consent. mRNA was extracted from EDTA blood after lysis; quantitative expression analyses of *FMR1, AKT, mTOR, S6K, FOXO3, FOXO1* genes were performed with specific TaqMan-Assays. Statistical analyses was performed with SPSS; statistical significance was set to P<0.05.

Main results and the role of chance: Gene expression levels of *AKT1, TSC2, mTOR, S6K, FOXO3* are significant higher in POI patients compared to controls (P< 0.009 or less).

The rate of *FMR1*-expression is highly correlated with *mTOR-, S6K* and *FOXO3*-expression levels (P<0.001) in all patients, in addition. When grouped according to ovarian reserve this effect is more pronounced in POI than in control patients. Additionally, the correlation of *FMR1* with *FOXO3* remained significant only in the POI subgroup.

The upregulation of AKT/mTOR-signaling in POI may reflect a compensative mechanism in POI aiming the activation of the last remaining follicles.

The linkage of *FMR1* with AKT/mTOR-signalling in peripheral blood comparable to prior results from germline, support its putative impact on the pathogenesis of POI and other folliculogenesis related disorders, such as poor ovarian response.

Limitations, reasons for caution: Results are based on limited patient numbers. More patients, stratified for age and other risks factors, are needed to further elucidate this mechanism.

Wider implications of the findings: This is the first evidence that *FMR1* is linked to an AKT/mTOR activation in POI potentially involved in its pathogenesis. Such a marker in peripheral blood offers a perspective towards its usability as a predictive tool in the diagnostics and prognosis of POI, if results are consistent in further studies.

Trial registration number: not applicable

O-081 High variability of molecular isoforms of AMH in follicular fluid and granulosa cells from human small antral follicles

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Study question: Is the composition of AMH isoforms different in follicular fluids (FF) and granulosa cells (GCs) from human small antral follicles?
Summary answer: There is a high viability of AMH isoforms in FFs and GCs. Even between same size follicles from the same women, the isoform composition differs.
What is known already: Anti Müllerian Hormone (AMH) is a member of the TGF-β superfamily produced by follicular granulosa cells (GCs) in women from late gestation to the end of reproductive live. AMH is suggested to inhibit aromatase (i.e. CYP19) expression and thereby decreasing the conversion of androgens to oestrogens in humans, especially in small antral follicles before dominance is achieved and thereby act as a gatekeeper of ovarian steroidogenesis. However, the exact function and processing of AMH in human follicles is still not clarified.
Study design, size, duration: This retrospective study measured AMH isoforms in human FF and GCs from small antral follicles using ELISA, Western blot, and immunofluorescence analysis. A total of 41 female adolescents and women aged 15 to 38 years (mean age: 29.7 years), who underwent ovarian tissue cryopreservation (OTC) at the University Hospital of Copenhagen, Rigshospitalet were included between year 2006 and 2020 included.
Participants/materials, setting, methods: Donated human ovarian medulla tissue were FFs and GCs were obtained in connection with OTC. The following isoforms were evaluated in FFs using ELISA analysis: full-length AMH precursor (proAMH), cleaved associated AMH ($AMH_{N,C}$), N-terminal pro-region (AMH_N), and active C-terminal (AMH_C) AMH. Antibodies specific for the N-terminal and the C-terminal AMH were used in both Western blot and immunofluorescence analysis of FFs and GCs.
Main results and the role of chance: A negative correlation between follicle diameter and the mentioned AMH forms were detected. Moreover, Western blot analysis detected various AMH forms in both FFs and GCs, which did not match the above-mentioned consensus forms suggesting an unknown proteolytic processing of AMH. The presence of these new molecular weight isoforms of AMH differs between individual follicles of identical size from the same woman.
Limitations, reasons for caution: The study group is limited and the significance of the variable AMH isoforms compositions between follicles cannot not be clarified from this data.
Wider implications of the findings: Collectively, these data suggest that intrafollicular processing of AMH is complex and variable, and thus, it may be difficult to develop an antibody based AMH assay that detect all AMH isoforms. Furthermore, the variability between follicles suggests that designing a proper standard will be difficult.
Trial registration number: not applicable

O-082 The ratio AMH/antral follicle count varies according to the etiologies of diminished ovarian reserve suggesting differences in follicular health

M. Grynberg[1], C. Lethielleux[2], V. Claire[2], I. Cedrin Durnerin[2], M. Peigné[2], S. Charlotte[3]

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Study question: Does diminished ovarian reserve (DOR) and its etiology impact the AMH/AFC ratio?
Summary answer: AMH/AFC ratio varies according to the etiology of DOR in young women, suggesting different impact on the follicular health, and further oocyte quality.
What is known already: Anti-Müllerian hormone and antral follicle count currently represent the two most accurate markers of the follicular ovarian status. Even though they may diagnose a reduction in the follicular stockpile, low values remain inefficient for predicting poor oocyte quality, in particular in young women. Since AMH is produced by the granulosa cells of follicles ranging from primary to small antral follicles, we hypothesized that the etiology of diminished ovarian reserve might differently impact the follicular health and their capacity of producing this peptide.
Study design, size, duration: From November 2018 to December 2021, we conducted a monocentric, retrospective study including a total of 484 infertile patients <37 years with DOR.
Participants/materials, setting, methods: All patients underwent measurement of AMH levels and AFC. DOR was diagnosed according to the Bologna criteria (AMH<1.1 ng/mL and AFC<7). AMH/AFC ratio was compared to values obtained in 154 tubal or male infertility patients matched for age and BMI, with AMH and AFC in the normal ranges. This ratio was studied according to the etiology of DOR: genetic (n=26), post-chemotherapy (n=102), idiopathic (n=215) or ovarian diseases (ovarian cyst or history of ovarian surgery, n=141).
Main results and the role of chance: Overall, median age of women with DOR was 30 (18-37) years. As expected, age and BMI were comparable in women with DOR and those having normal ovarian reserve tests. In addition, the AMH/AFC ratio failed to show any difference between these 2 groups (0.143 ± 0.22 vs. 0.166 ± 0.11, NS, respectively). Among women with DOR, the etiology was significantly associated with different AMH/AFC ratio. Indeed, patient with DOR of surgical origin (ovarian diseases group) displayed higher mean values (0.283 ± 0.32 ng/mL/ Foll) when compared with those included in genetic (0.079 ± 0.15 ng/mL/ Foll, p<0.01), idiopathic (0.103 ± 0.16 ng/mL/ Foll, p<0.03) or post-chemotherapy (0.084 ± 0.20 ng/mL/ Foll, p<0.01) groups. Moreover, genetic and post-chemotherapy DOR was also associated with lower AMH/AFC ratio in comparison with idiopathic DOR.
Limitations, reasons for caution: Despite interesting results, the retrospective nature of the present study may represent a limitation. Moreover, AMH/AFC ratio constitute an indirect method for assessing per follicle AMH production. We hypothesized that this ratio might reflect the follicular health. Its impact on natural conception and assisted reproductive technologies outcome is not known.
Wider implications of the findings: AMH/AFC ratio may represent an innovative tool aiming to indirectly assess follicular health and possibly oocyte quality in young women with DOR. The etiology of DOR differently impacts the follicular function as reflected by AMH/AFC ratio. Further data on live birth rates following natural or medically assisted pregnancies is needed.
Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS

SESSION 04: MORPHOLOGICAL EVALUATION FOR EUPLOIDY DETECTION

28 June 2021 Stream 3 10:00 - 11:30

O-083 Non-invasive, label-free optical analysis to detect aneuploidy within the inner cell mass of the preimplantation embryo

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Study question: Can we separate between control and reversine-treated cells within the inner cell mass (ICM) of the mouse preimplantation embryo by using label-free and non-invasive hyperspectral microscopy?
Summary answer: Hyperspectral microscopy is able to discern between control and reversine-treated cells using cellular autofluorescence in the complete absence of fluorescence tags.
What is known already: Embryo mosaicism (containing cells that are euploid (46 chromosomes) and aneuploid (deviation from the expected number of chromosomes)) affects up to 17.3% of human blastocyst embryos. Current diagnosis of aneuploidy in the IVF clinic involves a biopsy of trophectoderm (TE) cells or spent media followed by sequencing. In some blastocyst embryos these approaches will fail to diagnose of the proportion of aneuploid cells within the fetal lineage (ICM).
Study design, size, duration: The impact of aneuploidy on cellular metabolism was assessed by using cellular autofluoresence and hyperspectral microscopy (broad spectral profile). Two models were employed: (i) Primary human fibroblast cells with known karyotypes (4-6 independent replicates, euploid n= 467; aneuploid n= 969) and reversine induced aneuploidy in mouse embryos (5-8 independent replicates, 30-44 cells per group). Both models were subjected to hyperspectral imaging to quantify native cell fluorescence.
Participants/materials, setting, methods: The human model is comprised of euploid (male and female) and aneuploid (triploid and trisomies: 13, 18, 21, XXX, and XXY) primary human fibroblast cells. For the mouse model, we treated embryos with reversine, a reversible spindle assembly checkpoint inhibitor, during the 4- to 8-cell division. Individual blastomeres were dissociated from control and reversine treated 8-cell embryos. Blastomeres were either imaged directly or used to generate chimeric blastocysts with differing ratios of control:reversine-treated cells.
Main results and the role of chance: Following unsupervised linear unmixing, the relative abundance of metabolic cofactors was quantified: reduced nicotinamide adenine dinucleotide (NAD(P)H) and flavins with the subsequent calculation of the optical redox ratio (ORR: Flavins/[NAD(P)H + Flavins]). Primary human fibroblast cells displayed an increase in the relative abundance of NAD(P)H with a decrease in flavins, leading to a significant reduction in the ORR for aneuploid cells ($P < 0.05$). The mouse embryos displayed an identical trend as the human model between control and reversine-treated embryos. Mathematical algorithms were applied and able to distinguish between (i) euploid and aneuploid primary human fibroblast cells, (ii) control and reversine-treated mouse blastomeres and (iii) chimeric blastocysts with differing ratios of control and reversine-treated cells. The accuracy of these separations was supported by receiver operating characteristic curves with areas under the curve. We also showed that hyperspectral imaging of the preimplantation embryo does not impact on embryo developmental competence, pregnancy outcome and offspring health in a mouse model. We believe the role of chance is low as both human somatic cells and mouse embryos showed a consistent shift in cellular metabolism in response to human fibroblast cells that are aneuploid and reversine treated mouse embryos.
Limitations, reasons for caution: Further validation of our approach could include sequencing of the ICM of individual blastocysts to determine the proportion of aneuploid cells in ICM and correlate this with the metabolic profile obtained through hyperspectral imaging.
Wider implications of the findings: With hyperspectral imaging able to discriminate between (i) euploid and aneuploid human fibroblast cells and (ii) control and reversine-treated mouse embryos, this could be an accurate, non-invasive and label-free optical imaging approach to assess mosaicism within the ICM of mouse embryos, potentially leading to a new diagnostic tool for embryos**.**
Trial registration number: Not applicable

O-084 Computer vision can distinguish between euploid and aneuploid embryos. A novel artificial intelligence (AI) approach to measure cell division activity associated with chromosomal status.

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Study question: Can we distinguish between top-grade euploid and aneuploid embryos by AI measurement of cell edges in time-lapse videos?
Summary answer: Aneuploid embryos can be distinguished from euploid embryos by AI determination of a longer time to blastulation and higher cell activity.
What is known already: Continuous monitoring of the embryo development has brought out morphokinetic parameters that are used to predict pre-implantation genetic testing (PGT) results. Previous publications showed that euploid embryos reach blastulation earlier than non-euploid embryos. However, time-lapse data are currently under-utilized in making predictions about embryo chromosomal content. AI and computer vision could take advantage of the massive amount of data embedded in the images of embryo development. This is the first attempt to distinguish between euploid and aneuploid embryos by computer vision in an objective and indirect way based on the measurement of cell edges as a proxy for cell activity.
Study design, size, duration: We performed a retrospective analysis of 1,314 time-lapse videos from embryos cultured to the blastocyst stage with PGT results. This single-center study involved two phases; a comparison of the start time of blastulation between euploid (n=544) and aneuploid embryos (n=797). In phase two, we designed a novel methodology to examine whether precise measurement of cell edges over time could reflect cell activity differences in blastulation.
Participants/materials, setting, methods: We assumed that the delay in blastulation is reflected by higher cell activity that could be determined accurately for the first time using computer vision and machine learning to measure the length of the edges (from t2 to t8). We compared computer vision based measurements of cell edges, reflecting cell number and size, in videos of 231 top-grade euploid (n=111) and aneuploid (n=120) embryos.
Main results and the role of chance: The mean and standard deviation of blastulation start time was 100.1±6.8 h for euploid embryos and 101.8±8.2 h for aneuploid embryos ($p<0.001$). Regarding the measurement of cell activity, a computer vision algorithm identified the edges and provided a certainty score for each edge, higher when the algorithm is more certain that this is a cell edge (as opposed to noise in the images). A threshold was set to distinguish cell edges from noise using this score. The following results for top-grade embryos are shown as the sum of the edge lengths (μm) average of 160 pictures per embryo (frames between t2 and t8). The total length of the cell edges increased from two cells (420±85 μm) to eight cells (861±237 μm), in line with the mitosis events. Both the average total edge measured (450±162 μm for euploid embryos and 489±215 μm for aneuploid embryos, $p<0.01$) and the average total of the difference between consecutive frames (135±47 μm for euploid embryos and 153±64 μm for aneuploid embryos, $p<0.01$) were higher for aneuploid embryos than for euploid embryos. A regression model to differentiate between the two classes achieved 73% sensitivity and 73% specificity on this dataset.
Limitations, reasons for caution: The main limitation of this study is the difficulty to correlate our findings to other measure of cell activity. A more robust AI function (using not only cell edges lengths) would be required for future analysis to measure the cell activity in cell division up to the blastocyst stage.
Wider implications of the findings: Our results show for the first time that an AI based system can precisely measure microscopic cell edges in the dividing embryo. Using this novel method, we could distinguish between euploid and aneuploid embryos. This non-invasive method could further enhance our knowledge of the developing embryo.
Trial registration number: Not Applicable

O-085 In-depth analysis of embryo development: Differences among monosomic, trisomic and chromosomally chaotic embryos compared to euploid embryos.

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Study question: Is there any visible variation in the development of aneuploid embryos depending on the type of chromosome abnormality?
Summary answer: There were significant visible differences in the development of euploid, monosomic, trisomic and, especially, chaotic embryos.

What is known already: Aneuploidy rates are remarkably high in in vitro fertilized human embryos, with up to 50% of embryos diagnosed as aneuploid based on preimplantation genetic testing for aneuploidies (PGT-A). However, very little is known about the impact of specific aneuploidies during the early human embryo development. A recent publication showed that embryos with single chromosomal gain or loss reached the blastocyst stage later or earlier depending on the chromosome affected (Shahbazi et al., 2020). In our study, we wanted to detect observable differences in embryo behavior between embryos with different chromosomal abnormalities during the entire in vitro development.
Study design, size, duration: This was a retrospective study including 2,500 blastocysts with PGT-A results. Embryos were cultured in EmbryoScope systems until the fifth/sixth day of development (up to the time of trophectoderm biopsy). Automatic-annotations for division times and quality gradings were supervised routinely by senior embryologists using Guided Annotations Tool. Out of the total, 1,000 were euploid embryos used for reference and 1,500 were aneuploid embryos with one or more defects, including monosomic, trisomic and chromosomally chaotic embryos.
Participants/materials, setting, methods: Chromosome analysis was performed using next-generation sequence technology. Then, an in-depth analysis of time-lapse videos and supervised-automatic annotations was performed. We calculated the proportion of embryos, in each aneuploid category, that reached one specific event later than the expected value for euploid embryos plus one standard deviation. Later, we calculated the "relative risk" of an embryo of reaching the milestone late. We did the same for the time between milestones and for pairs of milestones.
Main results and the role of chance: Every aneuploid category was more likely to reach each specific embryo developmental event later than euploid embryos and the time gaps between developmental milestones were also statistically longer in aneuploid embryos ($p<0.0001$). The following results were the most interesting relative risks (RR) when we compared the proportion of embryos (in each aneuploid category) to the proportion of euploid embryos (RR for euploid=1). For reaching the division time to two cells (t2): 1.31 in monosomic embryos, 1.50 in trisomic embryos and 2.43 in chaotic embryos. For the division time to four cells (t4): 1.42 in monosomic embryos, 1.54 in trisomic embryos and 3.07 in chaotic embryos. For the division time to eight cells (t8) and the time of starting blastulation: 1.45 in monosomic embryos, 1.22 in trisomic embryos and 2.74 in chaotic embryos. Combined milestones were stronger indicators than each milestone by itself, the RR were: 1.63 in monosomic embryos, 1.81 in trisomic embryos and 3.35 in chaotic embryos for t2 and t4; 1.50 in monosomic embryos, 1.80 in trisomic embryos and 2.84 in chaotic embryos for t2 and t8; 1.46 in monosomic embryos, 1.90 in trisomic embryos and 3.43 in chaotic embryos for t4 and t8.
Limitations, reasons for caution: At this stage, we did not go down to specific chromosome abnormality as there were very few cases in each fully detailed category. Also, not all the embryos reached every developmental milestone.
Wider implications of the findings: Aneuploid embryos were significantly different from euploid embryos in the first five days of development. A large proportion of aneuploid embryos could be rejected because their developmental milestones falling outside the normal range. This could form part of an automated system for determining euploidy/aneuploidy from observation of embryos in vitro.
Trial registration number: 1902-VLC-018-MM

O-086 End-to-end deep learning for recognition of ploidy status using time-lapse videos

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Study question: Our Retrospective study is to investigate an end-to-end deep learning model in identifying ploidy status through raw time-lapse video.
Summary answer: Our deep learning model demonstrates a proof of concept and potential in recognizing the ploidy status.
What is known already: Since the time-lapse system has been introduced into the IVF lab, the relationship between morphogenetic and ploidy status has been often discussed. However, the result has not yet reached a united conclusion due to some limitations such as human labeling. Besides the statistical approach, deep learning models have been utilized for ploidy prediction. As such approaches are single image-based, the performance remains unpromising as previous statistical-based research. Therefore, in order to move further toward clinical application, better research design and approach are needed.
Study design, size, duration: A retrospective analysis of the time-lapse videos and chromosomal status from 690 biopsied blastocysts cultured in a time-lapse incubator (EmbryoScope+, Vitrolife) between January 2017 and August 2018 in the Lee Women's Hospital were assessed. The ploidy status of the blastocyst was derived from the PGT-A using high-resolution next-generation sequencing (hr-NGS). Embryo videos were obtained after normal fertilization through the intracytoplasmic sperm injection or conventional insemination.
Participants/materials, setting, methods: By randomly dividing the data into 80% and 20%, we developed our deep learning model based on Two-Stream Inflated 3D ConvNets(I3D) network. This model was trained by the 80% time-lapse videos and the PGT-A result. The remaining 20% has been tested by feeding the time-lapse video as input and the PGT-A prediction as output. Ploidy status was classified as Group 1 (aneuploidy) and Group 2 (euploidy and mosaicism).
Main results and the role of chance: Time-lapse videos were divided into 3-time partitions: day 1, day 1 to 3, and day 1 to 5. Deep learning models have been fed by RGB and optical flow. Combining 3 different time partitions with RGB, optical flow, and fused result from RGB and optical flow, we received nine sets of test results. According to the results, the longest time partition with the fusion method has the highest AUC result as 0.74, which appeared higher than the other eight experimental settings with a maximum increase of 0.17.
Limitations, reasons for caution: The present study is retrospective and future prospective research would help us to identify more key factors and improve this model. In addition, expanding sample size combined with cross-centered validation will also be considered in our future approach.
Wider implications of the findings: Group 1 and Group 2 approach provided deselection of aneuploidy embryos, while future deep learning approaches toward high mosaicism, low mosaicism, and euploidy will be needed, in order to provide a better clinical application.
Trial registration number: CS18082

O-087 Embryos with higher mitochondrial DNA ratios show better clinical outcomes in single euploid embryo transfer

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Study question: To assess whether there is a relationship between mitochondrial DNA content and implantation result.
Summary answer: The embryos with a higher mitochondrial DNA ratio increase pregnancy rate and implantation rate in single euploid embryo transfer.
What is known already: Mitochondria is an important organelle that generates energy during embryonic development. Recent literature points out that mitochondrial content and function may be related to implantation success and embryo viability. Some studies have linked increased ratios of mitochondrial DNA to aneuploidy, advanced maternal age, and euploid blastocyst with implantation failure, while others have failed to demonstrate similar findings.
Study design, size, duration: This study is a retrospective cohort study from 2016 to 2019, including 1465 single embryo transfer cycles.
Participants/materials, setting, methods: The involved embryos were biopsied on Day 5 or 6 and the mitochondrial DNA ratio of 1465 embryos was examined undergoing PGS/NGS. The mitochondrial DNA ratios were normalized for technical batch-to-batch variation. The mitochondrial DNA ratio between the implantation group and non-implantation group was statistically analyzed. Data were analyzed by the student's t-test for continuous variables and Chi-square test for categorical variables.
Main results and the role of chance: The mitochondrial DNA ratio of embryos was no significant difference between different age spans ($p=0.772$) and ploidy ($p=0.224$). D5 biopsied embryos, however, contained a significantly higher mitochondrial DNA ratio than D6 biopsied embryos ($p<0.0001$). All of the single embryo transferred embryos were classified into two groups; implanted and non-implanted embryos. Results from 1465 transferred embryos show that the mitochondrial DNA ratio of implanted embryos was statistically significantly higher than non-implanted embryos ($p=0.0053$). Besides, the cut-off

values were established, dividing the transferred embryos into high and low mitochondrial DNA ratio groups. The pregnancy rate and implantation rate of the high mitochondrial DNA ratio group was higher than the low mitochondrial DNA ratio group: [Pregnancy rate] 74% vs. 63.5% (p=0.0209); [Implantation rate] 57.3% vs. 50.8% (p=0.1907).

Limitations, reasons for caution: The mitochondrial DNA ratios were analyzed by bioinformatics processing in Miseq reporter software (Illumina) files in the BAM and FASTQ format. Not sure if there is reproducibility in different sequencing platforms.

Wider implications of the findings: There still remains a lack of clarity regarding the relationship between mitochondrial function and transfer outcome. This retrospective study links an association between increased mtDNA content and increased implantation.

Trial registration number: not applicable

O-088 Performance of a commercial artificial intelligence software for embryo selection (Embryoscope/KIDScore™) on predicting biopsied and non-biopsied blastocyst clinical pregnancy according to score subgroups.

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Study question: How informative is the score grade of KIDScore version 3 for day 5 blastocyst for clinical pregnancy in biopsied and non-biopsied embryos?

Summary answer: Potential clinical pregnancy is predicable according to score grades (above 7.0), regardless the use of PGT-A, in blastocysts on day 5.

What is known already: Time-lapse technology has promoted, along with the use of artificial intelligence (A.I.), a new spectrum of tools to improve embryo selection. Several software and algorithms have been launched in ART field in the last years, with the perspective of providing a substantial boost in IVF outcomes. KIDScore is one of these new tools, developed based on morphology and morphokinetics of embryo development with known clinical outcome and validated with transfer of blastocyst on day 3 or 5. Yet, it is highly recommended an in-house validation of any A.I. tool before it started to be apply in clinical decisions.

Study design, size, duration: Retrospective cohort study in a single private IVF center. Positive or negative clinical pregnancy (fetal heartbeat and gestational sac presence/absence) record of patient's autologous and donated cycles using fresh and frozen oocytes, with or without PGT-A embryos transfers using the Embryocope® Plus incubator, that underwent single embryo transfers (total sET, n=415; euploid=228, non-biopsied=187) of blastocysts developed on day 5 were included. Biochemical pregnancy and miscarriage were excluded of this analysis.

Participants/materials, setting, methods: Negative and positive clinical pregnancy KIDScore™Day 5's were stratified in three subgroups, according to V3 score intervals: subgroup 1: range between 1.0-3.9 (n=29), subgroup 2: 4.0-6.9 (n=154) and subgroup 3: 7.0-9.9 (n=232). sET of euploid embryos (n=228) were also analyzed in the described subgroups (subgroup 1: n=17; subgroup 2: n=93 and subgroup 3: n=118, respectively). For the analysis, Mann-Whitney, Chi-square and Fisher tests were used for statistical analysis, values of p<0.05 were considered significant.

Main results and the role of chance: Maternal age between overall positive and negative pregnancies were similar (38,48±3,86 versus 38,75±3,83,p=0,3573). When comparing score subgroups, overall positive clinical pregnancy rates were significant different [subgroup 1: 20.7% (6/29); subgroup 2: 43.5% (67/154); subgroup 3: 63.8% (148/232),p<0.0001]. When analyzing subgroup 1 versus subgroup 2 there was also a difference in positive clinical pregnancy (p=0.023) and subgroup 3 also showed a higher rate in clinical pregnancy when compared to subgroup 1 and 2 together (scores from 1.0 to 6.9,p<0.0001). Analyzing only euploid embryos, the results on positive clinical pregnancy were also significant different between subgroups [subgroup 1: 35.3% (6/17); subgroup 2: 45.2% (42/93); subgroup 3: 61.0% (72/118),p=0,024, and subgroup 1+2 versus subgroup 3,p=0,0115]. Maternal age between positive and negative clinical pregnancies in PGT-A cycles were similar (37,81±1,61 versus 38,38±3,25,p=0,069). Analyzing only non-biopsied embryos, the results on positive clinical pregnancy were also significant different between subgroups [subgroup 1: 0.0% (0/12); subgroup 2: 41.0% (25/61); subgroup 3: 66.7% (76/114),p=0,0343, and subgroup 1 + 2 versus subgroup 3,p<0.0001]. Maternal age between positive and negative clinical pregnancies in non-biopsied cycles were also similar (39,40±4,75 versus 39,22±4,43,p=0,7816). Positive clinical pregnancy in subgroup 3 were similar in biopsied and non-biopsied subgroups (61% versus 66.7%,p=0.4133).

Limitations, reasons for caution: The retrospective nature and low data of subgroup 1 (1.0-3.9 score), since they naturally are the last option to be chosen for transfer.

Wider implications of the findings: Differences on positive clinical pregnancy between subgroups (mainly scores greater than 7.0) reinforce the use of A.I. as a complementary tool for embryo selection. Interestingly, positive clinical pregnancy in 7.0-9.9 subgroup were similar in euploid and non-biopsied embryos, strengthening another potential application of A.I. in transposing embryo aneuploidy barrier.

Trial registration number: Not Applicable

SELECTED ORAL COMMUNICATIONS
SESSION 05: GENETIC ANALYSES IN ANDROLOGY

28 June 2021 Stream 4 10:00 - 11:30

O-089 A Genome Wide Association Study in men with unexplained infertility identifies nine SNPs at the FSHB locus to be associated with Follicle Stimulating Hormone level

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Study question: Which single nucleotide polymorphisms (SNPs) are associated with Follicle stimulating hormone (FSH) levels in men with unexplained infertility and can affect FSH action and spermatogenesis?

Summary answer: We identified a genomic region at chromosome 11p.14.1, including nine SNPs, that are significantly associated with FSH levels in men with unexplained infertility.

What is known already: FSH action is essential for the initiation and maintenance of human spermatogenesis. One well-studied SNP, *FSHB* c.-211G>T (rs10835638), is associated with *FSHB* mRNA transcription and directly affects FSH serum levels, testicular volume and spermatogenesis. Carriers of a T-allele in this promoter are diagnosed with functional secondary hypogonadism with isolated FSH deficiency.

Other genetic variants, for example at the *FSHR* have been shown to slightly modulate FSH action, however the clinical impact in these variants seems to be low. The so far identified FSH-associated SNPs revealed an impact of up to 2.3 % on FSH serum level variance.

Study design, size, duration: A Genome wide association study (GWAS) was performed on a clinically well characterized cohort of 742 men with unexplained infertility (discovery study). Of the nine identified SNPs, validation was performed for rs11031005 and the already described rs10835638 in an independent cohort of 1123 men with unexplained infertility (validation study).

Participants/materials, setting, methods: Patients were retrospectively selected from our CeRA database Androbase® applying strict selection criteria; DNA was isolated from stored EDTA-blood samples. Informative genetic

variants were identified using Illumina PsychArray v1.3. Illumina®GenomeStudio v2.0, PLINK v1.90 and R 3.6.3 were used to perform quantitative association analysis based on normalized FSH values. The validation study was performed using TaqMan PCR for SNP detection and R 3.6.3 for quantitative association to analyze the impact of each SNP on FSH level.

Main results and the role of chance: Imputation of the GWAS data revealed 94 SNPs with suggestive significance ($p < 8.56e-06$) and nine SNPs (including rs10835638) with genome-wide significance ($p < 4.28e-07$). The nine SNPs are all located at the *FSHB* locus on Chromosome 11p.14.1 and are in high linkage disequilibrium (LD). The validation study of 1123 patients with unexplained infertility for the SNPs rs11003005 and rs10835638 revealed a significant association with FSH ($p = 4.71e-06$ and $p = 5.55e-07$) and FSH/LH ratio ($p = 2.08e-12$ and $p = 6.4e-12$).The nine significant SNPs accounted for 3.60 – 4.65 % variance in FSH serum level each in the entire discovery cohort. In an oligozoospermic subgroup (n=249) this effect was increased to 4.89 – 6.95 %.

This the first GWAS in men with unexplained infertility. This study shows that not one single SNP, but rather a genomic region has an impact on FSH serum level in men with unexplained male infertility. This effect is even more pronounced in the more severe phenotype of oligozoospermic men.

Limitations, reasons for caution: The study is restricted to men with unexplained infertility, which might cause a selection bias. Validation and functional evaluation of the eight newly identified SNPs in independent cohorts would emphasize the results more. The sample size of 742 limits detection of loci with smaller effect on FSH levels.

Wider implications of the findings: The determination of one of the nine SNPs can improve diagnostic precision in identifying men with secondary functional hypogonadism with isolated FSH deficiency. An oligozoospermic subgroup of these men would putatively benefit from FSH treatment and has to be proven in randomized controlled trials.

Trial registration number: German Research Foundation CRU326

O-090 Correcting a PLCζ mutation in the human germ line to overcome hereditary infertility

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Study question: Can clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 gene editing result in the correction of a single base pair substitution that causes male infertility?

Summary answer: CRISPR/Cas9 administration during intracytoplasmic sperm injection (ICSI) leads to correction attempts of mutant phospholipase C zeta (PLCζ), howeverc loss-of-heterozygosity (LOH).

What is known already: Failed fertilization after ICSI can be caused by mutations in the sperm-related oocyte factor PLCζ which can be overcome by assisted oocyte activation (AOA). In this way, children may inherit the infertility-causing mutation. Mutation transmission can be overcome through CRISPR/Cas9 delivery during ICSI. In previous studies using CRISPR/Cas9 in the human germline for mutation correction, loss-of-heterozygosity (LOH, loss of the allele of one of the parents) was observed. Two different explanations were given, namely partial or complete paternal chromosomal loss or the correction of the mutation by using the maternal wild-type allele instead of the exogeneous supplied repair template.

Study design, size, duration: We injected a gRNA-Cas9 protein complex to target the PLCζ mutant allele, a repair template harboring the desired nucleotide substitution and an additional synonymous variant to track template usage, together with patient's sperm. To overcome fertilization failure, AOA was applied during ICSI. After a culture period of maximal 6 days the embryos were collected. At day 3, some embryos were dissociated in individual blastomeres. The extracted DNA was analyzed through different genetic sequencing techniques.

Participants/materials, setting, methods: Donated sperm of a patient experiencing complete fertilization failure after routine ICSI, harboring a heterozygous base pair substitution in *PLCZ1* (c.136-1G>C), was utilized. Sperm was injected in donated *in vitro* matured oocytes or *in vivo* matured oocytes containing clusters of smooth endoplasmic reticulum. Next-generation sequencing was used to assess correction potential. Short tandem repeat (STR) and single nucleotide polymorphism (SNP) assays were used to determine whether the sperm contained the mutation and to evaluate LOH.

Main results and the role of chance: CRISPR/Cas9 injections had no significant impact ($p > 0.05$) on embryonic development. Due to the heterozygous nature of the mutation, 47% (27/58) of the embryos originated from mutated sperm injection. The CRISPR components showed a high specificity with absence of insertions/deletions in 97% of the embryos originating from wild-type sperm (n=31). Embryos originating from mutant sperm (n=27) fall into three categories:(1) 22% showed the untargeted mutant allele, (2) 52% showed additional mutagenesis and (3) 26% showed the wild-type allele, which could be explained by correction. Mosaicism, defined as various editing events, was present in 17% (1), 21% (2) and 71% (3) of the embryos. The low occurrence of the synonymous variant, incorporated in the repair template, suggests that the template is not used during correction attempts. In only 29% (2/7) and 14% (1/7) of the 'corrected embryos', respectively long (>18Mb) or medium width LOH (4Mb) was observed through STR analysis. SNP analysis in closer proximity showed in 71% (5/7) of the embryos LOH, even in the absence of LOH through STR, suggesting also the occurrence of short width LOH. These results will be studied in more detail before definitive conclusions can be made. Chromosomal LOH will be studied by ddRADseq.

Limitations, reasons for caution: The occurrence of mosaicism and LOH might complicate the use of traditional CRISPR/Cas9 in human embryos and should be studied in detail to draw definite conclusions on its potential future use. To this end, genomic data have been produced from both individual blastomeres and whole-embryos which will be further analyzed.

Wider implications of the findings: Our findings demonstrate caution to use CRISPR/Cas9 to correct mutations in the germ line. They seem to contradict other reports that show predominant lack of mosaicism and presence of long width LOH. A deeper evaluation will be undertaken to define the length and type of LOH in this study.

Trial registration number: Not Applicable

O-091 Semen microbiota in patients with astenozoospermia and healthy controls: cluster analysis of real-time PCR data

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Study question: What are the differences in the semen microbiota composition of patients with asthenozoospermia and normospermia according to cluster analysis of PCR data?

Summary answer: The detection rate of 4 stable semen microbiota clusters and the dominant bacteria groups varied in patients with asthenozoospermia and normospermia.

What is known already: Most of the research dedicated to analyzing normal and pathological semen microbiota is based on 16S rRNA gene specific Next generation sequencing (NGS). It has shown that microbiota is represented by polymicrobial communities (clusters) that consist of microorganisms from different genera and bacteria phyla. Despite it being highly informative, NGS has several weaknesses: complex sample preparation, difficult sample intake control, long analysis process, complicated results interpretation, high cost of equipment and reagents. These factors make it virtually impossible to use this approach in routine medical practice. Quantitative real-time PCR (RT-PCR) is far more suitable for this.

Study design, size, duration: Patients included in the study (n=301) came to the "Garmonia" Medical Center (Yekaterinburg, Russia) either seeking preconception care or for infertility treatment. Depending on the spermiogram results, they were divided into two groups. Group 1 (n=171) — asthenozoospermia, Group 2 (n=130) — normospermia.

Participants/materials, setting, methods: Semen microbiota was analyzed using RT-PCR kit Androflor (DNA-Technology, Russia). Cluster analysis was performed for 201 samples with the total bacterial load (TBL) of at least 10^3 GE/ml (asthenozoospermia=96, normospermia = 105). Cluster analysis was

conducted using the k-means++ algorithm, scikit-learn. The Silhouette index and the Davies–Bouldin index (DBI) were used to confirm the stability of clusters.
Main results and the role of chance: Both in the samples with normospermia and asthenozoospermia, four stable microbiota clusters were distinguished. Cluster I was characterized by the prevalence of obligate anaerobes, *Lactobacillus spp*. were prevalent in Cluster II, Gram-positive facultative anaerobes were prevalent in Cluster III, *Enterobacteriaceae/Enterococcus spp.* were prevalent in Cluster IV. Cluster I was detected the most often in both groups. However, in normospermia it was represented by various obligate anaerobes without pronounced quantitative predominance of any bacteria group. In samples with asthenozoospermia one of the bacteria groups were prevalent in Cluster I: *Bacteroides spp./Porphyromonas spp./Prevotella spp., Peptostreptococcus spp./Parvimonas spp.* or *Eubacterium spp.* In samples with asthenozoospermia Cluster II was characterized by the prevalence of *Lactobacillus spp*., while in samples with normospermia other bacteria groups were present along with lactobacilli, mainly obligate anaerobes. In samples with normospermia *Corynebacterium spp.* and *Streptococcus spp.,* typical of normal microbiota of male UGT, were prevalent in Cluster III. In samples with asthenozoospermia Cluster III were characterized by the prevalence of *Staphylococcus spp.* In samples with asthenozoospermia *Lactobacillus spp* was present in Cluster IV along with *Enterobacteriaceae/Enterococcus spp.*, which was not typical of the samples with normospermia.
Limitations, reasons for caution: Cluster analysis was not conducted for the samples with TBL lower than 10^3 GE/ml, since their results were incompatible with the data received for the negative control samples.
Wider implications of the findings: Further research could determine the detection rate of the described bacterial clusters in semen with other pathologies. Establishing the relationship between the characteristics of semen microbiota and infertility in men might allow the development of new algorithms for treating patients with reproductive disorders, depending on the composition of semen microbiota.
Trial registration number: not applicable

O-092 Sperm phenotype, ICSI outcome and genetic diagnosis in case of severe asthenozoospermia with multiple morphological abnormalities of the flagellum

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Study question: What are the feasibility and outcome of ICSI in case of presumably genetic severe asthenozoospermia with Multiple Morphological Abnormalities of the Flagellum (MMAF phenotype)?
Summary answer: ICSI outcome for couples with MMAF phenotype does not differ from that of other couples requiring ICSI, regardless to the genetic etiology
What is known already: Severe asthenozoospermia, especially when associated with multiple morphological abnormalities of the sperm flagellum (MMAF phenotype), results in male infertility. Recent findings confirm that a genetic etiology is frequently responsible for this phenotype. In such situations, pregnancies can be obtained using ICSI. However, few studies have provided detailed analyses of the flagellar ultrastructural defects underlying this phenotype, of its genetic etiologies and of the results of ICSI in such cases of male infertility.
Study design, size, duration: We performed a retrospective study including 25 infertile men showing severe asthenozoospermia associated with a MMAF phenotype identified through standard semen analysis. These men were recruited from an academic center for Assisted Reproduction in Paris between 2009 and 2017. Transmission electron microscopy (TEM) and Whole Exome Sequencing (WES) were performed in order to precise the sperm ultra-structural phenotype and identify causal mutations, respectively. Twenty of the 25 patients benefited from assisted reproductive therapy by ICSI.
Participants/materials, setting, methods: MMAF patients were recruited based on reduced sperm progressive motility and increased frequencies of absent, short, coiled or irregular flagella, in comparison with fertile control men. A quantified analysis of the ultrastructural defects was performed for the MMAF patients and for fertile control men. ICSI results for the MMAF patients were compared to those of 528 ICSI attempts performed for non-MMAF individuals considering the sperm parameters and the distribution of ultrastructural axonemal anomalies.
Main results and the role of chance: Thorough categorization by TEM analysis of the flagellar anomalies found in these patients brought important precisions about the structural defects underlying asthenozoospermia and sperm tail abnormalities detectable through standard microscopy. In particular, absence of the central pair of axonemal microtubules was the predominant anomaly, observed significantly more frequently than in control men ($p<0.01$). Exome sequencing performed for 24 of the 25 patients (96%), identified in ten of them homozygous or compound heterozygous mutations that were described to be pathogenic (CFAP43, CFAP44, CFAP69, DNAH1, DNAH8, AK7, TTC29, MAATS1). A majority of those patients (55.5%,5/9) displayed the most severe ultra-structural defects of the axoneme. Forty ICSI attempts were performed for 20 MMAF patients. A hypo-osmotic swelling (HOS) test was required in 13 cycles (5 couples). Fertilization rate in MMAF group (65.7%) was not statistically different from the rate obtained for non-MMAF patients (66.0%) and did not differ according to the flagellar phenotype, nor to the use of HOS test, nor to the genotype. Clinical pregnancy rate per embryo transfer did not significantly differ between the MMAF group (23.3%) and the ICSI control group (37.1%). To date, 11 healthy babies were born among 20 MMAF patients.
Limitations, reasons for caution: The outcome of ICSI procedure was retrospectively assessed on a small sample and may be susceptible to recall bias. Moreover, TEM analysis was not available for some of the patients due to too low sperm concentration, and WES results are not yet available for all men included.
Wider implications of the findings: Couples requiring ICSI for presumably genetic severe asthenozoospermia should benefit precociously from appropriate phenotypic and genetic investigations. So far ICSI results appear similar to those observed in other ICSI indications. Identifying a genetic etiology and its mode of inheritance allows providing to these couples a most often reassuring genetic counseling.
Trial registration number: Not applicable

O-093 Male translocations in recurrent pregnancy loss: Natural conception versus PGD treatment: what is the right option?: A systematic review and meta-analysis.

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Study question: Does PGD treatment in couples with a history of RPL due to male translocations improve the outcome, increasing LBR and reducing miscarriage rate and time taken to live birth?
Summary answer: Live birth rate is significantly increased, miscarriage rate is significantly reduced using PGD. Time taken to achieve live birth rate is shorter in PGD treatment.
What is known already: Reciprocal translocation are the most common structural rearrangement in infertile men. The specific chromosomes and breakpoints involved might play an important role, often expressed as abnormal semen parameters or repeated pregnancy loss (RPL). The genetic counselling of these men remains challenging. Previous studies and meta-analysis performed showed no difference in live birth rate when comparing natural conception versus PGD treatment. However, the difference in miscarriage rate and time to live birth between PGD and natural conception has not been reported before in the medical literature.

Study design, size, duration: A systematic review of the literature was conducted through MEDLINE, EMBASE, and the Cochrane database up until December 2020. A comprehensive search yield 287 articles, 25 of which were included for abstract reading, finally, six were included in the meta-analysis.

Participants/materials, setting, methods: The six selected articles, reported on Live birth rate (LBR), miscarriage rate and time to live birth (TTLB) for natural conception compared to PGD for the same cohort of patients. All of the included articles were of retrospective design. The primary outcome was the comparison in LBR and the second outcome was the analysis in miscarriage rate and TTLB in the PGD group versus natural conception.

Main results and the role of chance: A total of 1438 couples that conceived naturally, had a LBR of 22.46%, compared with 43,17% among 681 couples that underwent PGD (0.53 95% CI (0.43-0.65) p o< 0,00001). The six articles included in this meta-analysis had significant homogeneity (I^2=96%). Comparison of miscarriage rates, natural conception represented 1339 miscarriages out of 1836 pregnancies, in comparison with 44 miscarriages out of 558 pregnancies achieved through PGD. The OR showed a 10 fold increase risk of miscarriage when conceiving naturally in couples with a male translocation (10.18; 95% CI (2.88-36.04) p=0.0003).

Regarding TTLB, the difference was not statistically significant, however it did reflect that PGD patients will have a shorter TTLB (3.56 95% CI (-0.88-8.00) p=0.12). One of the studies included, took into account the waiting list to access PGD funding, prolonging therefore the TTLB in the PGD group.

Limitations, reasons for caution: The main limitation of this study is the low number of studies. TTLB should be interpreted with caution given that one of the articles included the time of the waiting lists. More studies could demonstrate a shorter time period for these couples to conceive and have a successful ongoing pregnancy.

Wider implications of the findings: First study to demonstrate the value of PGD in decreasing miscarriage rates in couples with RPL. Specially when counselling couples with history of RPL with male translocations. PGD should be offered in these couples to improve the outcome, and to diminish the physical, emotional and sequelae of RPL and TOP.

Trial registration number: not applicable

O-094 Utilization of ultrastructural analysis and genomics of spermatozoa to better characterize subtle forms of male factor infertility

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Study question: Can sequencing the sperm genome provide insight into the various forms of male factor infertility caused by sperm organelle ultrastructural defects?

Summary answer: A comprehensive genomic assessment of spermatozoal DNA is able to identify genetic causes of ultrastructural defects visualized by transmission electron microscopy (TEM).

What is known already: To evaluate a man's reproductive potential, a conventional semen analysis through the assessment of concentration, motility, and morphology can indicate the proficiency of male gametes. Among those, conventional morphology assay can only provide indirect information on the different components of the sperm cell. The assessment of nanoscopic details such as chromatin, centriolar, mitochondrial, and axonemal components can only be observed by TEM. Indeed, TEM has been used to identify defects in the acrosome, chromatin compaction, and axonemal/periaxonemal structures. Furthermore, exome sequencing of spermatozoal DNA may identify novel causes and candidate genes for these ultrastructural defects.

Study design, size, duration: In the past 2 years, 20 men with history of fertilization failure or severe astheno-/terato-zoospermia were selected for TEM analysis of their spermatozoa, while 3 fertile men served as controls. Sperm head characteristics, intactness of fibrous sheath, and axonemal/periaxonemal structure were examined by diagnostic TEM. For consenting patients, NGS assessment was concurrently performed to identify mutations responsible for the structural abnormalities identified by TEM.

Participants/materials, setting, methods: TEM was performed on the ejaculates of 20 infertile patients and 3 fertile controls. Post-centrifugation cell pellets were resuspended, fixed, and dehydrated to be infiltrated and embedded onto the resin. Fixed specimens were sliced by ultramicrotome to 100-nm sections, then viewed by JEOL-1400 electron microscope at 300,000X magnification. At least 100 spermatozoa were evaluated by TEM. For consenting patients, DNA was extracted and amplified from at least 500 spermatozoa for concurrent NGS analysis.

Main results and the role of chance: Four types of sperm ultrastructural defects were observed, including globozoospermia, dysplasia of fibrous sheath (DFS), proximal centriole defect, and primary ciliary dyskinesia (PCD). One combined case of globozoospermia and DFS was identified. In globozoospermic patients (n=13), 97-100% of the spermatozoa displayed characteristic spherical heads with absence of acrosomes, dispersed chromatin, and perinuclear theca deformities. Centrosomal and axonemal structures were conserved. NGS identified gene deletions (DPY19L, PICK1, SPATA16) directly related to the globozoospermic phenotype. In patients with DFS (n=4), complete absence of flagellum was observed in 90-100% of spermatozoa. These defective gametes also displayed mitochondria disorganization, microtubular deformities, and cytoplasmic residues containing coiled flagellum with deformed capitulum within the plasma membrane. Contrary to the globozoospermia, acrosomes and nuclei appeared normal, indicating incomplete late spermiogenesis. Indeed, NGS confirmed gene deletions involved in flagellar development/function (AKAP4, SPAG16, CATSPER1). For the patient with proximal centriole defect (n=1), sperm nucleus, fibrous sheath, and flagellar structure were conserved. However, 90% of proximal centrioles assessed exhibited microtubular disorganization, confirmed by ODF2 mutation per NGS. In the PCD patient (n=1), chaotic flagellar microtubule arrangement and absence of outer dynein arms were prevalent in 90% of axonemal cross-sections examined, which was explained by a DNAH5 gene deletion.

Limitations, reasons for caution: While TEM can overcome the limitations of conventional semen analysis by providing direct visualization of the inner organelle arrangement of spermatozoa to accurately diagnose rare sperm pathologies, it is not routinely applied in clinics due to its high cost and technical specifications. Therefore, confirmatory NGS can provide additional diagnostic value.

Wider implications of the findings: Ultrastructural analysis with a concurrent genomic assessment characterized phenotypes and genotypes of rare sperm pathologies in infertile men. The utilization of TEM, corroborated by genomic assay, is therefore crucial for clinical and translational reproductive medicine to better characterize male factor infertility.

Trial registration number: N/A

INVITED SESSION

SESSION 06: FRONTIERS IN HUMAN EMBRYOLOGY

28 June 2021 Stream 1 11:45 - 12:45

O-003 The puzzling unknowns of abnormal fertilization and first cleavage

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Abstract text

Fertilization is a critical event in development in that it provides the connection between the gametes and the earliest stages of embryogenesis. Yet, despite the central importance of this process in contributing to embryo developmental fate, clinical embryologists have historically assessed fertilization merely by the number of pronuclei and, if two are present, perhaps, by the presence of two polar bodies. Even though over 20 years ago, time lapse imaging was applied for defining early events of fertilization (Payne et al., 1997), it is only with contemporary time-lapse imaging systems in the last few years that detailed evaluation of spatial and temporal events of fertilization have been described (Iwata & Yasuyuki, 2016; Cottichio et al., 2018). These careful analyses allow us to describe typical and atypical events of fertilization and how they are each associated with timing of the first cleavage division and subsequent embryo development.

In this lecture, we will first describe the fundamental underpinnings of fertilization and highlight the normal events associated with this process. We will then discuss gross morphological abnormalities as visualized by light microscopy and highlight the unknowns associated with these events. Finally, we will focus on time-lapse imaging studies, which have revealed the remarkable spatial and temporal coordination of meiotic resumption, pronuclear dynamics, chromatin organization and cytoplasmic/cortical modifications that occur during fertilization and the implications of aberrations for the first cleavage division.

At the conclusion of this presentation, attendees should be able to: Review the normal events associated with fertilization and the first cleavage division.

1. Describe gross morphological aberrations of these two fundamental processes.
2. Discuss temporal and spatial abnormalities in the coordinated sequence of events that underly these processes.
3. State the potential application of these abnormalities as predictors of abnormal embryo development.
4. Summarize the puzzling unknowns that underly these abnormalities.

Cottichio G, Mignini Renzini M, Novara P, Lain M, De Ponti E, Turchi D et al. Focused time-lapse analysis reveals novel aspects of fertilization and suggests new parameters of embryo viability. Hum Reprod 2018; 33(1): 23-31.

Iwata K, Yasuyuki M. Observation of human embryonic behavior in vitro by high-resolution time-lapse cinematography. Reprod Med Biol 2016; 15: 145–154.

Payne D, Flaherty SP, Barry MF, Matthews CD. Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography. Hum Reprod 1997; 12(3): 532–541.

O-004 Self-correction in human preimplantation development: What do we know?

A. Campbell[1]

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Abstract text

Recent advances in preimplantation genetic testing for aneuploidy (PGT-A) and time-lapse imaging have improved our understanding of the early human embryo confirming the variable patterns of development and chromosomal status. Aneuploidy is common and increased sensitivity in PGT-A allows the non-binary reporting of euploid-aneuploid mosaicism. The PGT-A result is the inference of the biopsied embryo's ploidy status at a point in time, by assessment of a small percentage of cells, and, whilst concordance with the rest of the embryo is high; it is not absolute.

Many reports have demonstrated that, with the transfer of embryos with increasing severity and complexity of mosaicism, comes compromised implantation, reduced ongoing pregnancy rates and increased miscarriage rates. Segmental mosaic embryos have been reported to have slightly reduced implantation potential compared with euploid counterparts. However, complex mosaic embryos are widely reported to result in severely reduced implantation success, if transferred.

Outside of PGT-A treatment cycles, undoubtedly fertility clinics are unwittingly transferring mosaic and aneuploid embryos daily, with variable success. The transfer of embryos in which mosaicism has been detected, although associated with lower implantation and higher miscarriage rates than euploid embryos, can lead to normal pregnancies and healthy births. We know that the placenta can harbour chromosomal aberrations which are absent from the fetus, and there are few reports of births with demonstrably high levels of mosaicism through fetal development. This raises the question as to whether correction mechanisms exist. In other words, do conceptuses become chromosomally more normal as development progresses, and what are the mechanisms, if so?

PGT-A, time lapse, novel live cell imaging and *in vitro* model techniques have enabled a more detailed study of early embryo development and consideration of the phenomenon of self-correction. This has provided insights and hypotheses surrounding the mechanisms of development and of self-correction.

The relatively lower levels of chromosome abnormality in the blastocyst, compared with cleavage stage, are well documented and indicative of some form of correction. A recent investigation reported that a large proportion of embryos initially diagnosed as mosaic were later diagnosed as euploid when assessed at day 12 of development; providing evidence of the depletion of abnormal cells throughout the early post-implantation stages.

There are many time-lapse reports of anomalous 'direct' or multichotomous blastomere divisions being associated with aneuploidy, and leading to developmental arrest or reduced implantation potential and of temporal delays in aneuploid embryos compared with their euploid counterparts. It is possible, therefore, that errors and even attempts to repair them, in individual cells in the rapidly developing embryo; which involve complex biochemical systems, could delay karyo- and cytokinesis, resulting in these detectable delays. The embryonic mortality model suggests that there is selection against embryos based on their degree of aneuploidy, such that aneuploid cell lines are lost during implantation.

We know that irregularities in blastomere cleavage can generate chromosome segregation errors but these may sporadically be confined to cells excluded, or extruded, from the morula or from the blastocyst; a possible exhibition of the clonal depletion or embryo mortality model.

The trisomic/monosomic rescue model suggests that aneuploid cells can give rise to diploid cells (and possibly uniparental disomy) through mitotic chromosome losses or gains. We know that an abnormal number of pronuclei does not always produce an aneuploid blastocyst and that early embryos exhibiting multinucleation can result in healthy live births. Finally, the preferential allocation of aneuploid cells to the trophectoderm model is based on the hypothesis that euploid cells are preferentially retained in the ICM in order to achieve viability.

This presentation aims to consider what we know, and discuss the theories and available evidence for self-correction.

INVITED SESSION

SESSION 07: EXCHANGE SESSION - IFS/ISAR: MULLERIAN ANOMALIES & ART

28 June 2021 Stream 2 11:45 - 12:45

O-005 Mullerian anomalies overview

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Abstract text

Mullerian Duct Anomalies- An Overview

Prof. Sudha Prasad

President Indian Fertility Society, Director, Matritava Advanced IVF & Training Centre, New Delhi, India

Congenital anomalies of the mullerian duct system is one of the complex disorder encountered in gynecological practice. Mullerian ducts are paired embryological structures which undergo fusion and resorption in utero to form the uterus, fallopian tubes, cervix and upper two-thirds of the vagina. Disruption in the mullerian duct development throughout embryogenesis could result a large spectrum of inherent abnormalities identified as mullerian duct anomalies (MDAs).

There is a wide variation in the prevalence of MDAs across various studies, ranging from 1–10% in the general population to 2–8% among infertile women and 5–30% among women with a history of miscarriage. These discrepancies in the reported prevalence are mainly attributed to lack of a universal classification system.

Different varieties of malformations can occur when this system is not well developed. It ranges from absence of uterus, cervix or vagina, septum/duplication of vagina to of the uterus and vagina to minor uterine cavity abnormalities. Mullerian malformations are frequently associated with abnormalities of the renal and axial skeletal systems. Therefore, on initial examination of MDAs patients, these points should be kept in mind.

Most mullerian duct anomalies (MDAs) are associated with functioning ovaries and age-appropriate external genitalia. These abnormalities are often recognized after the onset of puberty. After the onset of puberty, young women often

present to the gynecologist with menstrual disorders. Late presentations include infertility and obstetric complications.

A meta-analysis of nine studies comprising 3805 women with congenital uterine anomalies reviewed the obstetric outcome. The study reported that canalization defects such as septate and partial septate uteri had reduce fertility and increase rates of miscarriage and preterm delivery. None of the unification defects (bicornuate, unicornuate and didelphic uteri) reduce fertility but some are related to miscarriage and prematurity. Arcuate uteri are specifically associated with second-trimester miscarriage. All uterine abnormalities increase the risk of fetal malformation during delivery[1].

A retrospective longitudinal study concluded that reproductive performance of the unicornuate and didelphys uteri was poor (20–30% chance of carrying a pregnancy to term), while that of the septate and bicornuate uteri (live birth rate of 62%) was better than expected. The arcuate uterus had no impact on reproductive performance of women[2].

Agenesis of uterus and vagina requires surgical techniques, such as the Vecchietti and McIndoe procedures, have enabled many women to have normal sexual relations. Uterine transplant has changed the perspective of all other surgical advances and assisted reproductive technologies to improve fertility and obstetric outcomes[3, 4,5].

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O-006 Diagnosis debate – USG vs endoscopy

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[2]KJIVF and laparoscopy center - delhi, Reproductive medicine, Delhi, India

Abstract text

MULLERIAN ANOMAIES – DEBATE USG OR ENDOSCOPY

Mullerian duct anomalies are a complex spectrum of congenital anomalies resulting from defective fusion or canalization leading to different uterine anomalies. Early detection and proper diagnosis of uterine anomalies are paramount for proper management. Outflow obstruction defects like transvers septal defects or non canalised functional horn present early with complaint of pain while rest of patients present with amenorrhoea , infertility, repeated first-trimester abortion, fetal intrauterine growth restriction, and obstetric complications.The prevalence of uterine malformations is variable depending on the population studied , 0.4% , 4 % respectively in the general population and in infertile women while a high prevalence between 3 and 38% is reported in patients with repeated spontaneous miscarriages.

Imaging plays an important role in diagnosis and treatment planning in mullerian duct anomalies. There are different imaging and endoscopic modalities that can be used for the diagnosis and confirmation of uterine malformations. All modalities are having limitations and one need to select and combine various modalities depending on the clinical presentation of patient and pelvic examination. In younger patients or acute cases, trans abdominal ultrasonography (US) is the preferred method because it is readily available, inexpensive, and rapid and does not use ionizing radiation. However it may not give the complete picture because of poor demarcation especially in fatty patient and owing to complex nature of defects , Field-of-view restrictions with US, patient body habitus, and artefact from bowel gas. Pelvic magnetic resonance imaging (MRI) is an excellent tool in the diagnosis of Mullerian duct anomalies due to high soft tissue resolution. But it is more expensive and less available. 3D ultrasound may be a valid alternative to pelvic MRI as It is less expensive and better tolerated by patients however in doubtful cases of complex nature , hysteroscopy combined with laparoscopy may be considered to confirm the diagnosis. Another advantage of endoscopy is the opportunity to correct the defect in the same sitting in most of the cases.

Hysterosalpingography (HSG) and hysteroscopy are considered good modalities to assess the uterine cavity. Hysteroscopy provide the direct visualisation of the defect and considered as gold standard for cavity evaluation in doubtful cases of septate and bicornuate uterus and for simultaneous correction. However outer contour cannot be visualised so one need to use laparoscopy for complete evaluation which is a major drawback. Three-dimensional transvaginal sonography provides image quality like those provided by MRI and is being extensively used for diagnosis of all sorts of mullerian defects. it has got the advantage of realtime imaging which is helpful in distorted pelvic anatomy , visualisation of outer contour is possible ,which is considered very important to differentiate between bicornuate and septate uterus and unicornuate uterus with rudimentary horn. however it may not be possible in all cases to get a definitive diagnosis inspite of using a high end 3D machine specially in presence of artefacts , distorted contour and retroverted uterus. In such cases both modalities including MRI and endoscopy may be required to reach to a definitive diagnosis.

It can be concluded that primary imaging tool is still 2d ultrasound but 3D TVS should be included in all suspected anomalies along with complete careful pelvic examination to corroborate the findings of USG. In doubt ful or complex cases, MRI should be performed particularly for cervical and vaginal atresia and septum. endoscopy should be reserved for all doubtful cases for confirmation and for acute cases where a corrective surgery can also be planned to relieve the distress.

O-007 Do Mullerian anomalies need surgical correction

P. Trivedi[1]

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O-008 Improving outcomes in women with Mullerian anomalies

J. Malhotra[1], N. Malhotra[1], N. Malhotra[1]

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Abstract text

Mullerian Anomalies are present in approximately 5% to 7% of the general population and the incidence is a little more in infertile and recurrent miscarriage women. Most of the recent studies have reported that the obstetric outcome is compromised in this group with greater risk of infertility, recurrent pregnancy loss, intrauterine growth retardation, preterm birth and many other obstetric complications, which may be individually related to the different types of Mullerian Anomalies. In this presentation, We are going to discuss on how the outcomes are different in the various Mullerian Anomalies depending upon the degree of the defects related to different complications with more profound defects. We will also discuss on how to optimize the pregnancy outcomes with various interventions and what the literature review supports.

Trial registration number:
Study funding:
Funding source:

SELECTED ORAL COMMUNICATIONS

SESSION 08: ETHICS OF ART: OF EMBRYOS, OOCYTE DONORS AND RCTS

28 June 2021 Stream 3 11:45 - 12:45

O-095 Is the process to retract fabricated randomised clinical trials in reproductive medicine working sufficiently?

B.W. Mol[1]

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Study question: How do journal editors and publishers respond on randomised clinical trials in reproductive medicine that have been identified as fabricated?
Summary answer: Despite clear proof of fabrication, only a small minority of fabricated RCTs is retracted within 12 months.
What is known already: Randomised controlled trials (RCTs) are recognised as scientific investigations that have the least potential for bias and are therefore widely used to direct clinical practice. The validity of data in RCTs matters to the accountability of medical practice and the wellbeing of patients. Detection of integrity problems and subsequent action is therefore of imminent importance. Across all fields of medicine, it takes on average 4 years for papers labelled with research misconduct to be retracted. While this is partially explained by the time needed to detect the misconduct, the process of investigation and retraction is also slow and bureaucratic.
Study design, size, duration: We studied the articles of 4 authors who have published 52 clearly fabricated RCTs in obstetrics/gynaecology. Data fabrication was clear from duplicate baseline and outcome tables in studies on different interventions done in different patients in different periods. The duplications could be from the author themselves, or from other articles. Our findings were published in the public domain for 3 of the 4 authors, with an article on the fourth author being submitted.
Participants/materials, setting, methods: After detection of the fabrication, we approached authors and their institutes for an explanation. As a satisfying explanation was not given, we notified the editors of the involved 14 journals in February 2020. The universities where two of the authors were awarded a PhD were informed in August 2020. Here we compare the journals' response to Committee on Publication Ethics (COPE)-guidelines, we report the percentage of retracted papers and other responses of editors and publishers.
Main results and the role of chance: Two articles had already been retracted prior to our notification. Twelve months after we had notified the editors, 4/50 (8%) (1 journal) of the articles had been retracted, 3 (6%) (2 journals) were formally investigating with notification on their website, 6/50 (8%) (3 journals) were informally investigating (without visible notification), 3/50 (6%) (1 journal) had made an expression concern and stated "caution that clinical practice or guidelines should not be based on this report" without formal retraction, and one (2%) had investigated original data and cleared it (although numerous data were identical to a study published 10 years earlier). For the other 33 articles (11 journals) no visible action had been taken. None of the journals provided feedback to the whistle-blower as required by COPE. The University of Utrecht and the Vrije Universiteit Brussel had not taken any action against the awarded fraudulent PhDs.
Among the reactions of editors were the statements "I have been in the business long enough; It exists in all specialties and in every country", "we receive 80 submissions a week, I am too busy to respond on this" and "we did still not get an answer about the result of the investigation by the local court".
Limitations, reasons for caution: This was a sample of four authors who had published 52 articles. There might be investigations ongoing that are not visible for the outside world.
Wider implications of the findings: Retraction of fabricated studies is seldom happening, and a majority of journals is not following COPE. This not only puts patients at risk, but it also lets whistle-blowers down and it jeopardises the trustworthiness of research. COPE-regulations consider the interests of authors and publishers, but not the interests of patients.
Trial registration number: Not applicable

O-096 The ethics of stem cell-based embryo-like structures: a focus group study on the attitudes of Dutch professionals and lay citizens

A. Pereira Daoud[1], **W. Dondorp**[1], **A. Bredenoord**[2], **G. De Wert**[1]

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[2]*Utrecht University Medical Center, Medical Humanities, Utrecht, The Netherlands*

Study question: What are main themes guiding the attitudes of Dutch professionals and lay citizens with regard to the creation and research use of embryo-like structures?
Summary answer: The attitudes of Dutch citizens indicate feelings of trust and distrust, distinct 'embryo' conceptualizations, competing moral values and beliefs, and criteria for adequate regulatory safeguards.
What is known already: Researchers are hopeful that the creation and research use of so-called embryo-like structures (ELS), i.e., stem cell-based models that can mimic (parts of) early (human) embryogenesis, may provide a morally less controversial way of studying the period during which most human etiologies rise while also reducing and replacing the use of animals and/or human embryos in research. Scholars in the humanities and social sciences underline that public engagement and support will be essential in harvesting this presumed moral advantage. Studies on the public endorsement of and attitudes towards synthetic embryology remain nevertheless significantly scarce.
Study design, size, duration: For this qualitative study with a cross-sectional design, data were collected through four semi-structured focus group discussions (N = 33) between August and September 2020.
Participants/materials, setting, methods: The focus group interviews consisted of one pilot interview (n = 5), two interviews with lay citizens (n = 21), and one interview with professionals (n = 7). The pilot participants and lay citizens were invited based on sociodemographic characteristics of the Dutch public. Professionals were selected from the networks of the authors based on their experience and affinity with ethical and legal debates on emerging biotechnologies. The transcriptions were analyzed using inductive thematic analysis.
Main results and the role of chance: From our analysis emerged four themes: (1) *trust, distrust and ambivalence in synthetic embryology*, mainly due to concerns about scientific compliance with societal norms, also in view of a projected future ability of using ELS for reproductive ends; (2) *diversity of ELS-conceptualizations*, also in terms of their (non-)distinction from 'natural' human embryos; (3) *grounds for moral value and moral standing*, in which a possible potential to grow into a human being and perceived tinkering with nature were considered key issues of moral concern; and (4) *conditions for responsible embryo-like research*, with an emphasis on legally binding the use of ELS to non-reproductive research purposes. Interestingly, whereas questions on the developmental potential of ELS were critical for both the attitudes of professionals and those of lay citizens, lay citizens were much more concerned that synthetic embryology could fundamentally change human existence for the worse. In particular, lay citizens worried that synthetic embryology could lead to dystopian futures, for instance, because it stimulates a 'makeable human existence' or because of a presumed inability to monitor and control scientific progress. These findings may imply a correlation between fear of research and degrees of (un)familiarity with the governance systems of science.
Limitations, reasons for caution: Qualitative research methods provide rich and in-depth data for an ethical analysis of motivations and intuitions, but do not allow generalizations of the findings to broader publics.
Wider implications of the findings: The results may provide a useful starting point for further discussion and analysis of adequate regulatory parameters for the creation and research use of ELS. The findings also show that societal dialogue and public consultation can play a significant role in addressing concerns about emerging (bio)technologies.
Trial registration number: not applicable

O-097 The presentation of medical risks and incentives in egg donation: an analysis of Belgian, Spanish and UK fertility clinic websites

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[3]*De Montfort University, Centre for Reproduction Research, Leicester, United Kingdom ;*
[4]*Goethe-Universität Frankfurt am Main, Institut für Soziologie, Frankfurt am Main, Germany ;*
[5]*Consejo Superior de Investigaciones Cientificas, Institute of Public Goods and Policies, Madrid, Spain*

Study question: How do fertility clinics' websites of the UK, Belgium and Spain present the medical risks of egg donation and incentives?
Summary answer: Spanish and UK websites typically included more incentives to recruit egg donors compared to the Belgian websites. OHSS was overall the most discussed risk.
What is known already: People commonly turn to the internet for initial information. Primary presentations of information of a subject co-determine how an individual interprets the topic and thus influence later decisions. Considering the growing demand and clinics' dependency on egg donors, some scholars have expressed concerns that clinics might (initially) misrepresent risks to recruit more egg donors. Offering appealing incentives may also encourage potential donors to dismiss possible risks and side-effects.
Therefore, it is important to see how incentives (both monetary and non-monetary rewards) and risks are presented on the websites of fertility clinics, the first source of information for egg donors.
Study design, size, duration: This study is part of the EDNA-project, a multi-phased comparative study (2017-2021), that aims to explore the social, political, economic and moral configuration of egg donation in the United Kingdom, Belgium and Spain. In this study, we only focused on the medical risks of egg donation and incentives presented on the fertility clinics' websites of the three countries.
Participants/materials, setting, methods: We analysed the websites of all Belgian fertility clinics (n=18), and a maximum variation sample in the UK (n=21) and Spain (n=23). The sampling was based on the geographical location of the clinic, size/number of cycles performed each year, the clinic's status (independent or part of a larger clinical group) and whether the clinic was public or privately funded in the UK. Frame analysis and content analysis were used for analysis in Nvivo12.
Main results and the role of chance: No misrepresentation of risks was found. There was an extensive variety in the representation of risks. There were differences *between the websites of the three countries* (e.g. the risks of having acne was only discussed on Spanish websites), but also remarkable differences *within the websites of a particular country* (of the 40 risks, 13 were one-off mentions, each found on a single website). A description of a risk was generally accompanied by a minimization or normalization of the risk, or a statement about the fertility clinic's excellent care for their egg donors. These three approaches were often combined.

Our analysis differentiated between incentives (i.e. external rewards) and emotional appeals. An appeal to emotions (e.g. empathy) can also motivate behavioural action (e.g. donation) but was not considered as an incentive since there is no external reward.

While Belgian websites used almost no incentives, Spanish and UK websites used gratitude and a rewarding experience as incentives. However, only Spanish websites used free medical tests as incentives, while UK websites were the only ones that used discounts received with egg sharing as an incentive. All countries' websites used emotional appeals by enticing feelings of empathy for the recipients.
Limitations, reasons for caution: Not all UK and Spanish fertility clinics' websites were analysed. However, our international team of researchers applied a maximum variation sampling strategy. This generated samples of clinics per country that were as diversified as possible.
Wider implications of the findings: The study shows that incentives are more often used on the UK and Spanish websites than on the Belgian websites. All three countries' websites used emotional appeals. It should be studied how effective these incentives and emotional appeals are, and if there is a correlation with the potential donors' risk-perceptions.
Trial registration number: not applicable

O-098 Embryo selection using Artificial Intelligence (AI): Epistemic and ethical considerations

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Study question: What are the epistemic and ethical considerations of clinically implementing Artificial Intelligence (AI) algorithms in embryo selection?
Summary answer: AI embryo selection algorithms used to date are "black-box" models with significant epistemic and ethical issues, and there are no trials assessing their clinical effectiveness.
What is known already: The innovation of time-lapse imaging offers the potential to generate vast quantities of data for embryo assessment. Computer Vision allows image data to be analysed using algorithms developed via machine learning which learn and adapt as they are exposed to more data. Most algorithms are developed using neural networks and are uninterpretable (or "black box"). Uninterpretable models are either too complicated to understand or proprietary, in which case comprehension is impossible for outsiders. In the IVF context, these outsiders include doctors, embryologists and patients, which raises ethical questions for its use in embryo selection.
Study design, size, duration: We performed a scoping review of articles evaluating AI for embryo selection in IVF. We considered the epistemic and ethical implications of current approaches.
Participants/materials, setting, methods: We searched Medline, Embase, ClinicalTrials.gov and the EU Clinical Trials Register for full text papers evaluating AI for embryo selection using the following key words: artificial intelligence* OR AI OR neural network* OR machine learning OR support vector machine OR automatic classification AND IVF OR in vitro fertilisation OR embryo*, as well as relevant MeSH and Emtree terms for Medline and Embase respectively.
Main results and the role of chance: We found no trials evaluating clinical effectiveness either published or registered. We found efficacy studies which looked at 2 types of outcomes – accuracy for predicting pregnancy or live birth and agreement with embryologist evaluation. Some algorithms were shown to broadly differentiate well between "good-" and "poor-" quality embryos but not between embryos of similar quality, which is the clinical need. Almost universally, the AI models were opaque ("black box") in that at least some part of the process was uninterpretable.

"Black box" models are problematic for epistemic and ethical reasons.

Epistemic concerns include information asymmetries between algorithm developers and doctors, embryologists and patients; the risk of biased prediction caused by known and/or unknown confounders during the training process; difficulties in real-time error checking due to limited interpretability; the economics of buying into commercial proprietary models, brittle to variation in the treatment process; and an overall difficulty troubleshooting.

Ethical pitfalls include the risk of misrepresenting patient values; concern for the health and well-being of future children; the risk of disvaluing disability; possible societal implications; and a responsibility gap, in the event of adverse events.
Limitations, reasons for caution: Our search was limited to the two main medical research databases. Although we checked article references for more publications, we were less likely to identify studies that were not indexed in Medline or Embase, especially if they were not cited in studies identified in our search.
Wider implications of the findings: It is premature to implement AI for embryo selection outside of a clinical trial. AI for embryo selection is potentially useful, but must be done carefully and transparently, as the epistemic and ethical issues are significant. We advocate for the use of interpretable AI models to overcome these issues.
Trial registration number: not applicable

INVITED SESSION
SESSION 09: DATA REPORTING SESSION
28 June 2021 Stream 1 14:00 - 15:00

O-009 Data from the ESHRE PGT consortium – year 2019

A. Van Montfoort[1], M. De Rycke[2], F. Carvalho[3], C. Rubio[4], F. Bronet[5], F. Spinella[6], V. Goossens[7]

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[6]Genoma Group srl, Molecular Genetics Laboratories, Rome, Italy ;
[7]ESHRE, Central Office, Grimbergen, Belgium

Abstract text

Study question: Which are the trends shown in data collection XXI of the European Society of Human Reproduction and Embryology (ESHRE) PGT Consortium compared with previous years?

Summary answer: Data collection XXI, year 2019, represents valuable data on PGT activity in (mainly) Europe and reports on the main trends observed, being the further expansion of comprehensive testing technology in PGT-SR and PGT-A.

What is known already: The ESHRE PGT Consortium was set up in 1997 and from that time has been collecting data on PGT and PGT-A. The PGT database comprises the world's largest collection of PGT / PGT-A data providing a valuable resource for data mining and for following trends in PGT practice. So far, up to the year 2015, data collections were carried out in a retrospective data way, from 2016 onwards a prospective data collection was in place.

Study design, size, duration: As the nature of PGT/ PGT-A treatments has changed significantly over the last years and IVF cycle management and genetic analysis techniques are getting more complex, ESHRE uses an online data collection system in which data are collected prospectively from oocyte retrieval to analysis, embryo transfer and pregnancy / live birth. Data are collected cycle by cycle on a voluntary basis.

Participants/materials, settings, method: For the 2019 data, individual centres (31) from 19 countries directly entered the data into the PGT database through software developed by ESHRE. Data were analysed at ESHRE headquarters and include all aspects of PGT/PGT-A cycles.

Main results and the role of chance: The Consortium has analysed the PGT analyses (n=2735) performed in 2019. The indications for PGT included inherited chromosomal abnormalities (n=253 analyses), monogenic disorders (n=1105 analyses), aneuploidy testing for infertility (n=1111 analyses) or combinations of the above (n=266 analyses). In addition, 662 clinical pregnancies and 216 deliveries have been analysed in detail. The methods used for biopsy were polar body (2%), cleavage stage biopsy (35%) and blastocyst biopsy (61%; comparable with data from 2018). The methodology used for diagnosis is what is evolving most over the last years, with data set XXI (2019) showing around 7% of FISH, 37% of PCR and 55% of WGA. Within WGA 90.6% of the analysis were done using NGS, in 4.4% cases SNP arrays were used and in 2.4% array-CGH was used. The overall clinical pregnancy rate is about 24% per analysis. The baby data show that it is difficult for most centres to have a detailed follow-up.

Limitations, reasons for caution: The findings apply to the 31 participating centres and may not represent worldwide trends in PGT. Data were collected prospectively, but details of the follow-up on PGT pregnancies and babies born were limited.

Wider implications of the findings: The ESHRE PGD Consortium continues its activities as an important forum for PGT practitioners to share data and exchange experiences. The information extracted from the data collections helps to monitor quality issues in PGT and survey the introduction and effectiveness of new PGT technologies and methods.

INVITED SESSION

SESSION 10: LOOKING BEYOND THE XX FACTOR

28 June 2021 Stream 2 14:00 - 15:00

O-010 The significance of male factor and sperm damage to the offspring

J. Kirkman-Brown[1]

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O-011 You are what your father ate- epigenetic implications

A. Soubry[1]

[1]KU Leuven, Dept. of Public Health and Primary Care\rFaculty of Medicine, Leuven, Belgium

Abstract text

Title: You are what your father ate: epigenetic implications.
Author: Adelheid Soubry
Abstract:
An unhealthy life style, obesity and excess of dietary fats or chronic consumption of processed foods create a harmful environment for sperm health. In the current presentation our most recent findings will be presented on male exposures related to an unhealthy life style and the effects measured on clinical sperm and/or embryo parameters in humans. Next, epigenetic implications will be shown from male obesity, "healthy" and "unhealthy" foods and other related determinants (such as advanced age) before conception. Our results lend support for the existence of epigenetic windows of susceptibility in life. If the acquired epigenetic signatures are passed down to the next generation(s) this may affect future health.

Our data are based on human studies, including the Newborn Epigenetics Study (NEST) cohort, The Influence of the Environment on Gametic Epigenetic Reprogramming (TIEGER) study and the Epigenetic Legacy of Paternal Obesity (ELPO) cohort.

In brief, we found that consumption of healthy food items, such as vegetables, fruits and nuts, is positively related to total motile sperm count (TMC), while consumption of fast foods (such as fries) is associated with lower TMC. Frequent consumption of fast foods (incl. pizza and fries) is associated with opposing effects on DNA methylation patterns at the DMRs of imprinted genes (such as *IGF2* and *MEG3-IG*), compared to dietary patterns rich in whole grains and vegetables. These results correspond to our findings in sperm from obese *versus* non-obese men, and are in line with our earlier findings in children from obese fathers. While this talk will be a compilation and comparison of our research findings, it will also serve as a base for guidance and counselling in infertility.

Our results fit our new concept of the Paternal Origins of Health and Disease (POHaD), where the role of the father has been suggested in disease development of his future offspring. If better understood, tailored dietary changes may positively shape the human sperm epigenetic profile and future programming of offspring health.

Trial registration number:
Study funding:
Funding source:

POSTER DISCUSSION

SESSION 11: REPRODUCTIVE EPIDEMIOLOGY, SOCIO-CULTURAL ASPECTS AND HEALTH ECONOMY POSTER DISCUSSIONS

28 June 2021 Stream 3 14:00 - 15:00

P-718 Paternal smoking in the preconception period is associated with an increased risk of spontaneous miscarriage in a dose-dependent manner: a systematic review and meta-analysis

N. Du Fossé[1], M.L. Van der Hoorn[1], N. Buisman[1], J. Van Lith[1], S. Le Cessie[2], L. Lashley[1]

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Study question: What is the association between paternal lifestyle factors in the preconception period and the risk of spontaneous miscarriage?
Summary answer: Preconception paternal cigarette smoking is associated with an increased risk of spontaneous miscarriage, while no associations were found with paternal alcohol consumption and obesity.
What is known already: Although maternal lifestyle risk factors for miscarriage are well-established, studies on potentially contributing paternal factors remain sparse. Recently, a significant association was found between advanced paternal age and spontaneous miscarriage. Biological evidence indicates that smoking, excessive alcohol consumption and obesity may lead to sperm oxidative DNA damage, being a known risk factor for miscarriage.
Study design, size, duration: Systematic review and meta-analysis.
Participants/materials, setting, methods: PubMed and Embase databases were searched in August 2020. Paternal factors examined were: cigarette smoking, alcohol consumption and Body Mass Index (BMI). A qualitative risk of bias assessment was performed for all included studies. Meta-analysis was performed if sufficient data was available from studies that controlled for maternal factors. PRISMA guidelines for systematic reviews were followed.
Main results and the role of chance: The systematic search included 3386 articles of which 11 articles met the inclusion criteria. In a meta-analysis of eight studies, paternal smoking of >10 cigarettes per day in the preconception period was found to be associated with an increased risk of spontaneous miscarriage, after adjustment for maternal smoking status (1-10 cigarettes per day: 1.01, 95% CI 0.97-1.06; 11-20 cigarettes per day: 1.12, 95% CI 1.08-1.16; >20 cigarettes per day: 1.23, 95% CI 1.17-1.29). Based on five available studies, no clear association was found between paternal alcohol consumption and spontaneous miscarriage. No studies were retrieved that evaluated the association between paternal BMI and spontaneous miscarriage.
Limitations, reasons for caution: Investigating the relation between paternal lifestyle factors and spontaneous miscarriage is challenging and prone to different forms of bias, especially in retrospective studies.
Wider implications of the findings: Awareness of the association between heavy paternal smoking in the preconception period and the risk of spontaneous miscarriage should be raised. More well-designed studies are needed to further investigate the effects of other paternal lifestyle factors on the risk of spontaneous miscarriage.
Trial registration number: not applicable

P-719 Self-declared infertility and child desire among women of reproductive age in the National Survey of Demography and Health, Brazil

S. Garcia[1], M. Koyama[2]

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Study question: This article aims to characterize from a socio-demographic point of view, women of reproductive age who wish to have children, declared themselves infertile, and their search for treatments and outcomes.
Summary answer: It is essential to develop specific population surveys on infertility in Brazil to identify its magnitude and main economic and social components.
What is known already: Commonly neglected in developing countries where public policy is incipient, infertility brings social, economic and psychological consequences to couples. It is considered as a serious public health problem whose impact varies among different populations and acquires relevance for specific communities. In Brazil, there are no clinical or demographic data that point us to the magnitude of the problem, its social characteristics and impact. Taking into account the postponement of motherhood for after 30 years, there will probably be an increase in the number of women and couples who may resort to infertility treatments to fulfil the desire for procreation.
Study design, size, duration: The National Survey of Demography and Health of Women and Children (PNDS) is a cross-sectional study and a household complex probabilistic sampling. The sampling units were selected according to a stratified model of simple random conglomerates in two stages: lottery draw and household draw. The last survey was conducted between June 2006 and May 2007 in 14,617 households. In the selected households, interviews were conducted with 15,575 women of reproductive age.
Participants/materials, setting, methods: The participants consisted of 15.575 women between 15 and 49 years, representative of the five Brazilian macro-regions. The information was obtained through questionnaires, applied in person, raising information on fertility, fecundity, contraception, use of health services and socioeconomic profile. The interviewer's team was formed by approximately 100 people and 27 supervisors, all-female, divided into nine regional teams. The system used for data entry was the Census and Survey Processing System - CSPro.
Main results and the role of chance: The survey results indicate that of women who wish to have children, 9.2% declared themselves infertile; 50,8% of them sought health services for treatment; non-black women had higher percentages of demand compared to black women (62.4% versus 41.3%). Also, there were higher percentages of seeking help from women belonging to classes A (61.2%), B (83.3%) and C (60.9%) compared to those belonging to classes D (30.4%) and E (7.8%) On the other side, almost half of women did not seek help to get pregnant (49,1%); this percentage is higher among black women (58%). Moreover, women in classes D and E had the highest percentages of non-demand, 69.6% and 92.2%, respectively. The reasons cited for those who do not seek help, are "I think there is no solution" (54,7%); "I don't think I can get help" (17.3%), "financial reasons" (26.8%) or "I don't know where to get it" (1,2%). Among those who sought help, 48,5% are under treatment, 24,4 % said there is no solution; 15,8% are waiting for service and 11,3% have no money for treatment. Significance limit was established for values of $p < 0.05$. The analysis was performed in the programs Stata v.9 and/or SPSS v.14.
Limitations, reasons for caution: The limitations of the study are recognized. Firstly, opinions are restricted to the moment of the interview and, thus, the desire for children may change over time. Secondly, the statement of infertility is based on self-declaration, not on clinical diagnosis.
Wider implications of the findings: This is the first study based on PNDS 2006 data on infertility and demand for treatments in Brazil. It can contribute to providing insights, raising new questions and discovering relevant categories and dimensions of analysis to be taken into account in future studies and surveys.
Trial registration number: not applicable

P-736 Elevated levels of ambient air pollutants increase the primary sex ratio in human embryos

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Study question: Are there any associations between ambient outdoor air pollution and the primary sex ratio (PSR)?
Summary answer: Short-term exposure to increased PM_{10}, $PM_{2.5}$ and NO_2 levels were significantly associated with higher PSR.
What is known already: PSR estimates represent a backward extrapolation from data based on spontaneous or induced abortions, fetal deaths or live births and are usually male-biased. A recent study, analyzing 3- to 6-day-old embryos derived from assisted reproductive technology (ART) procedures, showed that the sex ratio at conception is unbiased (0.5). Epidemiologic studies of air pollution on secondary (birth) sex ratio showed that higher levels of particulate pollution were associated with increased rates of female birth. However, a direct association between urban levels of air pollutants and PSR has not been reported.
Study design, size, duration: A retrospective cohort study was carried out to assess the impact of long- or short-term exposure to six ambient outdoor air pollutants (particulate matter, $PM_{10\mu m}$ and $PM_{2.5\mu m}$; SO_2; CO; NO_2; O_3) on PSR (XY/XX) of couples undergoing their first IVF cycle for preimplantation genetic screening (N=337). Data was from fixed air quality monitoring stations across the city between January 2014 and December 2018. Embryos with sex chromosome abnormalities were excluded from the analysis.
Participants/materials, setting, methods: Average concentrations of the pollutants for the 90 (long-term exposure) and 15 days (short-term exposure) predating oocyte retrieval represented the exposures of interest. Pollutant levels were categorized into quartiles (Q_1 to Q_4) and exposure risk was divided into two periods in which average concentrations and confidence intervals for the pollutants were in the upper quartile (Q_4 period) or not (Q_1-Q_3 period). The

strength association between exposure risk and PSR was performed through analysis of covariance.

Main results and the role of chance: The estimated means of PM_{10}, $PM_{2.5}$, SO_2, NO_2, O_3 and CO for Q_1-Q_3/Q_4 periods were 27.7/39.3, 16.7/23.7, 2.5/3.9, 37.0/46.4, 32.2/45.3 µg/m3 and 0.64/0.87 ppm and 26.3/43.0, 16.0/26.3, 2.4/4.2, 36.5/47.8, 31.7/50.4 µg/m3 and 0.62/0.90 ppm for long- and short-term exposures, respectively. PM_{10}, $PM_{2.5}$ and NO_2 levels in the Q_4 period had significantly higher PSR (138.1, 134.0 and 137.6) when compared to Q_1-Q_3 period (94.4, 98.1 and 96.4) for the short-term exposure (*p=0.0193*; *p=0.0439*; *p=0.0180*, respectively). PM_{10}, $PM_{2.5}$, SO_2, NO_2 and CO levels in the Q_4 and Q_1-Q_3 periods for the long-term exposure showed no significant effect on PSR. Contrastingly, O_3 levels in the Q_4 period had significantly lower PSR (82.6) when compared to Q_1-Q_3 (115.9) for the long-term exposure (*p=0.0202*). A monotonic increase in PSR was observed with increased PM_{10} concentration in the Q_4 period for the short-term exposure (F-ratio: 4.4476; *p=0.0352*).

Limitations, reasons for caution: Some limitations of the study should be underlined, such as its retrospective nature, exposure assessment based on pollutant levels derived from a network average across city sites, and limited extrapolation of the results to the general population.

Wider implications of the findings: Our data suggest that short-term exposure to environmental factors could affect the primary sex ratio in polluted seasons or cities. A monotonic effect on PSR in the case of exposure to increasing PM_{10} levels was identified.

Trial registration number: Not applicable

P-740 Socio-cultural and clinical implications of 'routine' AMH testing in India: Insights from an interview study with the healthcare professionals (HCPs)

P. Satalkar[1], V. Provoost[2]

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[2]Bioethics Institute Ghent, Department of Philosophy and Moral Sciences- Ghent University, Ghent, Belgium

Study question: How do Indian healthcare professionals describe their clinical experience with and perspectives on AMH testing in Indian women seeking fertility treatments including fertility preservation?

Summary answer: The HCPs cautioned against AMH testing as a screening tool in presumed fertile Indian women due to its anticipated impact on women's arranged-marriage prospects.

What is known already: AMH test is being increasingly used to assess women's ovarian reserve (OR) while planning fertility treatments or to guide decisions about fertility preservation (FP). There is weak evidence suggesting that serum AMH level and fertility treatment outcomes vary in different population groups. Surveys with women in reproductive age (e.g. the US, Ireland, the Netherlands) indicate that a majority wants to know their OR to aid reproductive decision making. As yet, both globally and in an Indian context, there are only few qualitative studies exploring the views of HCPs on the OR assessment in clinical practice and its socio-cultural implications.

Study design, size, duration: This paper reports the findings of an exploratory qualitative research aimed at understanding whether and how elective fertility preservation could influence reproductive autonomy of Indian women. Between June 2018 and April 2019, IVF specialists and obstetricians practicing in ten cities across five Indian states were interviewed in English (language commonly spoken) using a semi-structured interview guide. The discussion about OR assessment with AMH-testing was initiated by the participants indicating its significance in their clinical practice.

Participants/materials, setting, methods: The study sample included 17 male and 15 female HCPs, the majority (18/32) was practicing in Mumbai. Twenty-six of them were in private practice while six worked as OBGYNs in publicly funded teaching hospitals. Twenty-six participants were interviewed in their clinics and the remaining six using Skype or telephone. After several rounds of immersive reading, the interview sections on OR and AMH-test were analyzed inductively using Braun and Clarke's thematic analysis.

Main results and the role of chance: Several participants reported that many of their patients present with decreased OR (DOR) at a younger age and need higher dosages of hormones for ovulation induction compared to the dosages mentioned in international guidelines. They corroborated this experience with a few peer-reviewed articles indicating a six-years age difference in OR of Indian women undergoing IVF compared to Spanish women. A majority of participants advocated for the rational use of OR assessment in IVF patients but warned against its indiscriminate use or interpretation out of context due to concerns about overdiagnosis of ovarian factor infertility and overtreatment with IVF with donor eggs. Although the physicians who had performed elective FP perceived AMH test as a simple, affordable and empowering tool to guide FP decisions, most participants were critical of using AMH-test as a screening tool in young, presumed fertile women completing university education. They were concerned that a diagnosis of DOR as a result of such screening in this population in the Indian context will adversely impact women's chances of marriage and might further increase pressure on women to get married and complete their childbearing early even if they are not ready for it.

Limitations, reasons for caution: This is the first qualitative study assessing views of Indian HCPs on AMH testing. These results are indicative rather than a representation of views of Indian HCPs. Almost half of the contacted HCPs did not respond to interview requests; we do not know whether they had different views.

Wider implications of the findings: The insights on clinical implications of AMH testing in India are relevant to other societies beyond the Euro-American and Australian context where AMH testing will increase in the future. The socio-cultural implications of 'routine' AMH testing in India urges us to be aware of similar implications in other cultural contexts.

Trial registration number: Not applicable

INVITED SESSION

SESSION 12: RECURRENT IMPLANTATION FAILURE: A MULTI DISCIPLINARY APPROACH

28 June 2021 Stream 4 14:00 - 15:00

O-012 Diagnostic strategies

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Abstract text

Most discussions of the management of recurrent implantation failure (RIF) will start by expressing the criteria for 'diagnosis' of the condition. And here lies our first mistake. Defining RIF as a condition diagnosable in terms of a standard number of embryos transferred without achieving pregnancy suggests that is a medical diagnosis in itself, for which, like other diseases, an optimal treatment strategy exists. But the failure of our field to improve outcomes in patients who suffer RIF suggest that we have approaching the condition wrongly.

RIF is NOT medical disease diagnosed by applying simple clinical criteria. It is a personal experience suffered by patients and which can uncomfortably challenge their doctors. IN order to address the mistake we have made in our approach to RIF, we need to recognise that the condition needs to be recognized in a different way. In practical clinical terms, RIF can be considered to have occurred at the point when the patient(s) and their doctor conclude that further action is necessary beyond the standard work up and treatment approach for IVF.

Our failure to understand what constitutes RIF has also resulted in large and methodologically sound trials of interventions proposed to 'treat' RIF missing the target. Based as most have been on the premise that RIF is a disease for which the optimal treatment exists if we can just find it, these trials are largely destined to produce negative results, as those in whom the intervention was effective cannot be differentiated form those in whom it might have caused harm. The resulting 'zero sum' requires the study authors to write off the studied intervention as ' ineffective' potentially depriving certain patients of a useful treatment.

So how can we address the therapeutic impasse that we have created?

The answer is surely clear. When our standard best practices fail to result in pregnancy after IVF in a particular patient, we simply need to try to understand why. This requires the classic diagnostic approach: consider RIF a symptom; look for signs that might explain it and order investigations to pin down the cause(s).

So what diagnostic approaches should be applied? In many cases, the embryo factor will already have been subject to considerable scrutiny. When treatment continues to fail, the focus will therefore tend to turn at this point to the patients.

This is where the story begins to look more positive. In recent years it has become clear how active a role the endometrium plays in embryo implantation. As these novel endometrial factors have become elucidated, clinical tests of their function have begun to emerge. While many contributory factors merit investigation when RIF is considered to have arisen, this lecture will introduce the concept of the 'endometrial diagnostic toolkit' that promises to enable us to treat RIF on the basis of individual rationale rather than blind hope, and to test the efficacy of targeted rather than blind interventions.

O-013 Therapeutic aspects

POSTER DISCUSSION

SESSION 13: SAFETY AND QUALITY OF ART THERAPIES POSTER DISCUSSIONS

28 June 2021 Stream 1 15:15 - 16:30

P-751 Immediate versus postponed frozen-thawed embryo transfer after IVF/ICSI: a systematic review and meta-analysis

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Study question: Can frozen embryo transfer (FET) be offered immediately after a stimulated IVF/ICSI cycle without compromising live birth rate (LBR)?
Summary answer: FET in the menstrual cycle immediately following the stimulated IVF/ICSI cycle was associated with a slightly higher LBR compared to standard postponed FET.
What is known already: It is standard clinical practice to postpone FET for at least one menstrual cycle following a failed fresh transfer or a freeze-all cycle. This practice is thought to minimize any possible residual negative effect of ovarian stimulation, with excessive steroid levels and multiple corpora lutea, on the resumption of a normal ovulatory cycle and receptivity of the endometrium. Even so, elective deferral of FET is an empirical strategy based on suggestions rather than solid scientific evidence and may unnecessarily delay time to pregnancy, causing frustration and decreased quality of life to couples.
Study design, size, duration: Systematic review and meta-analysis according to PRISMA guidelines. Original studies on subfertile women aged 18-46 with any indication for treatment with IVF/ICSI investigating the timing of FET after IVF/ICSI were included. Intervention was defined as FET in the menstrual cycle immediately following the stimulated IVF/ICSI cycle. Comparator was defined as FET in the second or subsequent menstrual cycle following IVF/ICSI. Risk of bias was assessed using Robins-I and quality of evidence using GRADE.
Participants/materials, setting, methods: PubMed (MEDLINE) and EMBASE databases were searched for MeSH and Emtree terms, as well as text words related to timing of FET, up to March 2020. There were no limitations regarding year of publication or duration of follow-up but to English language. The primary outcome was LBR. Secondary outcomes were implantation rate, pregnancy rate, clinical pregnancy rate (CPR), time-to-pregnancy, miscarriage rate (MR), cycle cancellation rate and patient wellbeing.
Main results and the role of chance: Out of 4124 search results, 15 studies were included in the review. Studies reporting adjusted odds ratios (aOR) for LBR, CPR and MR were included in meta-analyses. All studies (n=15) were retrospective cohort studies involving a total of 6,304 immediate FET cycles and 13,851 postponed FET cycles including 8,019 matched controls. Twelve studies of very low to moderate quality reported no difference in LBR with immediate versus postponed FET. Two studies of moderate quality reported a statistically significant increase in LBR with immediate FET and one small study of very low quality reported better LBR with postponed FET. Trends in rates of secondary outcomes followed trends in LBR regarding timing of FET. The meta-analyses showed a significant advantage of immediate FET (n=2,076) compared to postponed FET (n=3,833), with a pooled aOR of 1.20 (95% CI 1.01-1.44) for LBR and a pooled aOR of 1.22 (95% CI 1.07-1.39) for CPR.
Limitations, reasons for caution: Limitations include the retrospective design and heterogeneity of studies included, limiting comparison and pooling of data. With little transparency regarding cancellation rates, the risk of selection bias is apparent. Further, confounding by embryo quality is a limitation. Small sample sizes are a limitation to subgroup meta-analyses.
Wider implications of the findings: The standard clinical practice of postponing FET for at least one menstrual cycle following a failed fresh transfer or a freeze-all cycle may not be best clinical practice. Randomized controlled trials including data on cancellation rates are highly needed to provide high grade evidence regarding clinical practice and patient counseling.
Trial registration number: not applicable

P-766 Neurodevelopment in fetuses conceived by assisted reproductive technologies following fresh and frozen embryo transfer

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Study question: Do *in vitro* fertilization (IVF) offspring present different neurodevelopment assessed by fetal neurosonography and infant neurobehavioral tests as compared to those spontaneously conceived (SC)?
Summary answer: IVF offspring, especially those obtained after fresh embryo-transfer (ET), showed subtle structural differences in fetal neurosonography and poorer neurobehavioral scores at twelve months of age.
What is known already: The number of pregnancies following assisted reproductive technologies (ART) is currently increasing worldwide. Concerns about the neurodevelopment of subjects conceived by IVF have been rising and mostly studied in children and adolescents with inconsistent results. Many of the identified risk associations were only observed in subgroups or disappeared after adjustment for covariates, mainly multiple pregnancy and gestational age at birth. It is unknown whether fetal brain development and cortical folding differ prenatally in IVF fetuses as compared to SC.
Study design, size, duration: This is the first study examining fetal neurodevelopment by neurosonography in IVF fetuses.

A prospective cohort study of 210 singleton pregnancies recruited from 2017 to 2020, including 70 SC gestations, 70 conceived by IVF following frozen ET (FET) and 70 IVF after fresh ET.

Fetal neurosonography was performed in all pregnancies. Additionally, *Ages & Stages Questionnaires* (ASQ) were obtained at 12 months of corrected age.
Participants/materials, setting, methods: IVF pregnancies were recruited from a single Assisted Reproduction Center, ensuring homogeneity in IVF stimulation protocols, endometrial preparation, laboratory procedures and embryo culture conditions. SC pregnancies were randomly selected from low-risk fertile couples and paired to IVF by maternal age. Fetal neurosonography including transvaginal approach was performed at 32±2 weeks of gestation, measured offline by a single investigator and normalized by biparietal or occipitofrontal diameter. ASQ were obtained postnatally, at 12 months of corrected age.
Main results and the role of chance: Study groups were similar and comparable regarding maternal age, body mass index, study level and employment rate together with exposure to smoke, alcohol, aspirin and corticoids during pregnancy, gestational age (32±2 weeks) and estimated fetal weight (1700±400g) at neurosonography.

As compared to SC pregnancies, both IVF populations showed differences in cortical development with reduced parieto-occipital (fresh ET 12.5mm [SD 2.5] vs FET 13.4 [2.6] vs SC 13.4 [2.6]), cingulate (fresh ET 5.8 [IQR 4.2-7.4] vs FET 5.8 [4.1-7.5] vs SC 6.5 [4.8-7.8]) and calcarine (fresh ET 13.5 [IQR 10.1-16.1] vs FET 14.5 [12.1-15.8] vs SC 16.4 [14.3-17.9]) sulci depth together with lower Sylvian fissure grading. Cortical development changes were more pronounced in the fresh ET group as compared to FET. Corpus callosum length and insula depth were lower in FET and fresh ET groups, respectively. Neurosonographic changes remained statistically significant after adjustment by ethnicity, gender, gestational age and weight centile at scan.

IVF infants showed worse ASQ scores, especially in fresh ET for communication, personal-social, fine-motor and problem-solving skills. Gross-motor scores were significantly lower in FET as compared to SC and fresh ET. Differences were statistically significant after adjustment by maternal ethnicity, study level, employment status, breastfeeding, gender and corrected age.

Limitations, reasons for caution: The reported neurodevelopmental differences are subtle, with most neurosonographic findings lying within normal ranges.

Infertility factors contribution to the outcome cannot be unraveled from the ART procedure itself.

The milder features found in FET individuals cannot condition the technique´s choice and must be considered together with their global perinatal results.

Wider implications of the findings: Neurosonography is an appropriate tool to identify subtle brain differences between fetuses exposed and not exposed to ART. Prenatal features were consistent with postnatal neurobehavioral findings. These results support the relevance of a neurodevelopmental follow-up in IVF patients. Further studies are warranted to assess the long-term performance in these subjects.

Trial registration number: not applicable

P-767 Cumulative live birth rate after IVF - trend over time and the impact of blastocyst culture and vitrification

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Study question: Has cumulative live birth rate (CLBR) improved over time and which factors are associated with such an improvement?

Summary answer: During 2007-2017, CLBR per oocyte aspiration increased significantly (27.0 % to 36.3 %), in parallel with an increase in blastocyst transfer and cryopreservation by vitrification.

What is known already: While it has been shown that live birth rate (LBR) per embryo transfer (ET) is higher for fresh blastocyst than for fresh cleavage stage embryo transfer, CLBR per oocyte aspiration, including one fresh ET and all subsequent frozen embryo transfers (FET), does not seem to differ between the two culture strategies.

Study design, size, duration: STUDY DESIGN, SIZE, DURATION: National register study including all oocyte aspirations performed in Sweden 2007-2017, n=124 700. Donation cycles excluded.

Participants/materials, setting, methods: Data were retrieved from the Swedish National Registry of Assisted Reproduction (Q-IVF). CLBR was defined as the number of deliveries with at least one live birth resulting from one oocyte aspiration, including all fresh and/or frozen embryo transfers within one year. The delivery of a singleton, twin, or other multiples was registered as one delivery. Cryopreservation of cleavage stage embryos was performed by slow freezing and of blastocyst by vitrification.

Main results and the role of chance: Overall, the CLBR per oocyte aspiration increased significantly during the study period, from 27.0 % to 36.3 % (OR 1.039, 95% CI 1.035-1.043) and from 30.0 % to 43.3 % if at least one ET was performed (AOR 1.055, 95% CI 1.050-1.059). The increase in CLBR was independent of maternal age, number of oocytes retrieved and number of previous IVF live births. The CLBR for women <35 years and ≥ 35 years both increased significantly, following the same pattern. During the study period a substantially increasing number of blastocyst transfers were performed, both in fresh and in FET cycles. An important contributor included in the blastocyst strategy, may be the extended culture of the total cohort of embryos, also embryos earlier discarded at early cleavage stages, in order to reach the blastocyst stage. These embryos may contribute to the total number of available blastocysts and thereby increase the chance of a live birth within that oocyte aspiration cycle. Other important predicting factors for live birth, such as number of embryos transferred, could not explain the improvement, on the contrary the single embryo transfer (SET) rate increased with time.

Limitations, reasons for caution: The retrospective design implicates that other confounders of importance for CLBR can not be ruled out. In addition, some FET cycles might be performed later than one year post oocyte aspiration for the last year (2017) and are thus not included in this study.

Wider implications of the findings: The results suggest that blastocyst transfer, particularly when used in FET cycles and in combination with vitrification, is an important contributor to the improved live birth rates over time. This gives a possibility for fewer oocyte aspirations needed to achieve a live birth and a shortened time to live birth.

Trial registration number: -

P-777 Comparison of GnRH-a trigger and GnRH-a plus low-dose HCG trigger for high ovarian responders in IVF/ICSI: A retrospective study based on propensity score matching

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Study question: Does GnRH agonist trigger for high responders during IVF/ICSI cycles improve the number of good-quality embryos, the incidence of moderate-to-severe OHSS, and pregnancy outcome compared to GnRH-a plus low-dose HCG?

Summary answer: GnRH-a trigger alone can effectively reduce the incidence of moderate-to-severe OHSS in women with high ovarian responses without affecting embryo quality.

What is known already: Previous studies have shown conflicting results on the different trigger protocol in high responders in IVF/ICSI outcomes, and as for women with high ovarian response, there is little known about the effects of GnRH-a plus low-dose HCG versus GnRH-a alone on oocytes maturation, the rate of good quality embryos, the incidence of moderate-to-severe OHSS, and pregnancy outcome during IVF/ICSI cycles.

Study design, size, duration: A retrospective analysis was conducted on patients with high ovarian response who received IVF/ICSI treatment with a flexible GnRH antagonist regimen, at the Center of Reproductive Medicine, Chengdu Jinjiang Hospital for Maternal and Child Health Care, from January 1 2017 to December 31 2018. Using 1:1 propensity score matching, 513 cases entered each group (a total of 1,026 females).

Participants/materials, setting, methods: The high responders were included and assigned to groups A (0.2 mg triptorelin) and B (0.2 mg triptorelin plus 2000 IU HCG) for final oocyte maturation. Their basic clinical characteristics, information about controlled ovarian stimulation cycle, embryologic data, and pregnancy outcome in FET were retrospectively compared. The main outcome measures of the study were the rate of good-quality embryos, the number of available embryos, the incidence of moderate-to-severe OHSS, and the cumulative live-birth rate.

Main results and the role of chance: Using 1:1 propensity score matching, 513 females were included in each group. No significant differences in baseline clinical data were found between the two groups, including age at diagnosis, spouse's age, the duration of infertility, the infertility type, and the cause of infertility, BMI, anti-Müllerian hormone (AMH) levels, and the antral follicle count (AFC) ($p > 0.05$). None significant differences were found in the total doses of gonadotropin (Gn), the duration of ovarian stimulation, serum P and LH levels on the trigger day, the number of oocytes retrieved, the rate of 2PN embryos, and the rate of good-quality embryos ($p > 0.05$). The serum E2 level on the trigger day in group A was significantly higher than that in group B ($p < 0.001$). Women in group A had a lower incidence rate of moderate-to-severe OHSS than individuals in group B ($p < 0.001$). There was a non-significant difference in the cumulative live-birth rate between the two groups ($p > 0.05$).

Limitations, reasons for caution: As this is a retrospective study that uses data initially collected for other purposes, limitations may exist in the selection,

implementation, and measurement biases that cannot be avoided. However, our study underlies the need for further prospective, multi-center joint-controlled studies to validate these findings.
Wider implications of the findings: This study demonstrates that GnRH-a alone can reduce the incidence of moderate-to-severe OHSS without harming embyro quality in women with high ovarian response. These findings need further prospective validations in hyperresponsive populations by multi-center, large-sample, randomized controlled studies.
Trial registration number: N/A

P-783 Clinical, obstetric and perinatal outcomes after vitrified-warmed euploid blastocyst transfer are independent of cryo-storage duration.

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Study question: Is cryo-storage duration associated with the outcomes after vitrified-warmed euploid single blastocyst transfer?
Summary answer: Lower live-birth-rates from blastocysts cryo-stored for periods longer than 3-months are mostly imputable to the worse quality of the embryos being warmed across sequential transfers.
What is known already: Blastocyst vitrification is crucial in modern IVF. Given its widespread application, a constant comprehensive monitoring of its effect on reproductive outcomes is pivotal. For instance, the effect of cryo-storage duration on embryo implantation potential, gestational and perinatal outcomes is object of a still ongoing investigation. The evidence in this regard are contrasting especially with regard to similar or decreased live birth rates among blastocysts subject to long-term cryo-storage. When investigating the neonatal outcomes, instead, no impact of blastocyst cryo-storage duration has ever been reported to date. Yet, data on euploid blastocysts and adjusted for quality and full-blastulation day are needed.
Study design, size, duration: Retrospective observational study. We included 2688 vitrified-warmed euploid single blastocyst transfers. The primary outcome was the live-birth-rates (LBR) according to cryo-storage duration clustered as ≤60, 61-90, 91-180, 181-360, 361-720, 721-1080 and >1080-days. The secondary outcomes were the miscarriage rate, the rates of gestational and perinatal issues among the deliveries, and the mean gestational age and birthweight among the babies born. All data were adjusted for confounders through linear or logistic regression analyses.
Participants/materials, setting, methods: We included all vitrified-warmed transfers (range:1-8) conducted between May-2013 and March-2020 by 1884 patients (age:38±3yr) undergoing one blastocyst stage PGT-A cycle and obtaining ≥1 euploid embryo at our private clinic. Among putative confounders, only the number of sequential transfer from the same patient, blastocyst quality (Gardner's scheme) and full-blastulation day (5-7) significantly associated with the LBR through univariate regressions. No association was reported for sperm factor, maternal age, incubator, and culture media.
Main results and the role of chance: The LBR of euploid blastocysts cryo-stored for ≤60-days was 49.4% (N=319/646) versus 48.7% (N=292/599; OR:0.98,95%CI:0.78-1.21,p=0.82) between 61-90-days, 42.9% (N=291/679; OR:0.77,95%CI:0.62-0.96,p=0.02) between 91-180-days, 41.7% (N=169/405; OR:0.73,95%CI:0.57-0.94,p=0.02) between 181-360-days, 34.7% (N=50/144; OR:0.55,95%CI:0.37-0.79,p<0.01) between 361-720-days, 53.4% (N=63/118; OR:1.17,95%CI:0.79-1.74,p=0.42) between 721-1080-days, and 50.5% (N=49/97; OR:1.05,95%CI:0.68-1.60,p=0.83) for >1080-days. However, when these data were adjusted for blastocyst quality and full-blastulation day, all the multivariate-OR were not-significant. Indeed, the longer the cryo-storage period the worse the quality of the euploid blastocysts transferred (e.g. AA-blastocysts were 74% among embryos cryo-stored for ≤90-days, but always <70% for embryos cryo-stored for longer periods, p<0.01; similarly, day5-blastocysts were ~50% among embryos cryo-stored for ≤90-days, but always <50% for embryos cryo-stored for longer periods, p=0.02). The miscarriage-rate (overall 14%, ranging 7-18%) was not associated with cryo-storage duration already from univariate regressions. Also the gestational (overall 6%, ranging 0-8%) and perinatal issues rates (overall 3%, ranging 0-5%) were not associated with cryo-storage duration already from the univariate regressions. Neither the gestational age nor the birthweight showed significant associations with cryo-storage duration, as confirmed by linear regressions. In fact the rate of newborns whose weight was normal-for-gestational-age was similar across all cryo-storage duration groups (overall 81%, ranging 80-83%).
Limitations, reasons for caution: The prevalence of first transfers decreases from ≥95% for procedures conducted ≤90-days from vitrification to 71%, 39%, 22% and 4% for procedures conducted between 91-180, 181-360, 361-720 and >720-days, respectively. However, also the sequential number of transfer was not associated with the LBR when adjusted for blastocyst-quality and full-blastulation day.
Wider implications of the findings: Cryo-storage by vitrification is confirmed safe in the hands of experienced operators, and its duration does not impact any outcome. This information is valuable for freeze-all cycles, but also for women cryo-preserving surplus embryos for second pregnancies; in this regard, 6.8% of the patients in this study delivered ≥2 LBs.
Trial registration number: not applicable

POSTER DISCUSSION

SESSION 14: REPRODUCTIVE ENDOCRINOLOGY POSTER DISCUSSIONS

28 June 2021 Stream 2 15:15 - 16:30

P-622 Prothrombotic biomarkers during controlled ovarian stimulation for assisted reproductive techniques

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Study question: Does the evolution of prothrombotic biomarkers over time differ between antagonist and long agonist stimulation protocols for assisted reproductive techniques (ART) ?
Summary answer: The hypercoagulable state was higher and persistent in the agonist and antagonist with hCG triggering groups compared to the antagonist with GnRH agonist triggering group.
What is known already: Controlled ovarian stimulation (COS) for ART is associated with supra-physiological serum estradiol levels, a hypercoagulable state and an increased risk of venous thrombosis. Most thromboembolic events associated with COS occur in the context of ovarian hyperstimulation syndrome (OHSS). The use of hCG for final follicular maturation increases the risk of OHSS. In antagonist protocols, GnRH agonist triggering is known to prevent or reduce OHSS and is therefore widely used in women at risk. The impact of the different IVF protocols on pro-thrombotic biomarkers is unknown.
Study design, size, duration: In this prospective observational cohort study, infertile women undergoing COS for ART in 2017-2019 at the University Hospitals of Geneva and Lausanne (Switzerland) were included. We evaluated changes in key coagulation parameters (D-dimers, factor VIII, fibrinogen activity, protein S and protein C) and thrombin generation, our primary outcome, (using 5 pM of tissue factor) by calibrated automated thrombinography before stimulation (T1), on the day of ovulation triggering (T2) and seven days after triggering (T3).
Participants/materials, setting, methods: COS was started without hormonal pre-treatment. Protocols were prescribed according to the standards used in each centre taking into account the risk of OHSS (agonist protocol with hCG trigger in women without OHSS risk (Group 1); antagonist protocol in women at risk of OHSS with hCG trigger (Group 2;) or GnRH agonist trigger

(Group 3;); variation of endogenous thrombin potential (ETP) was measured and compared among groups using mixed effects linear regression model.

Main results and the role of chance: A total of 64 women were included: 24 were in group 1, 16 in group 2, and 24 in group 3. The mean age (SD) was 37.8 (2.8), 35.9(5.2) and 34(4.6) years in groups 1, 2 and 3 respectively. As expected, women in group 1 had a statistically lower level of anti-müllerian hormone (p=<0.001), a lower antral follicular count (p=<0.001) and lower number of MII oocytes and embryos obtained (p=<0.001). Mean serum estradiol levels were 1836 (1160), 1628 (815) and 3754 (2165) ng/L at T2, and 945 (471), 1061 (495) and 413 (729) ng/L at T3, in group 1 to 3, respectively. In multivariable regression analysis, the levels in group 3 were statistically higher at T2 and lower at T3 (overall time*group interaction: p<0.001).

The mean ETP was similar between all groups at T1, and increased in all groups at T2 (1442, 1426 and 1486 nM/min in groups 1, 2 and 3, respectively) (p=0.013). Overall, ETP evolution over time was statistically different between groups, with the lowest increase of ETP between T1 and T3 in group 3. Protein C and protein S levels were stable, while D-dimers, fibrinogen and factor VIII increased at T2 and T3 in all groups.

Limitations, reasons for caution: Stimulation protocols were prescribed according to the clinical profile and OHSS risks; groups therefore differ substantially in regards to age and ovarian reserve. Thromboembolic events are rare events after COS, we therefore evaluated biological markers of hypercoagulability and not clinical events.

Wider implications of the findings: Women with GnRH agonist triggering protocol did not increase mean ETP in the week after ovulation, while women with hCG triggering did. This different prothrombotic profile was independent of the variation of the other coagulation parameters investigated. This effect of ovulation triggering should be confirmed by further studies.

Trial registration number: NCT04188444

P-631 Embryo euploidy rates following follicular or luteal start ovarian stimulation. A prospective study with repeated ovarian stimulation ovarian stimulation cycles.

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Study question: Is there any difference in embryo euploidy rates following luteal phase phase (LS) and follicular phase (FS) start ovarian stimulation.

Summary answer: The number of euploid blastocysts and embryo euploidy rate are comparable when comparing FS and LS.

What is known already: Random start ovarian stimulation (starting at any time of the cycle) has been traditionally used in women undergoing urgent fertility preservation for medical reason. Although there is accumulating evidence that in infertile women, LS can result in equivalent number of oocytes and embryos as compared with FS, no study has evaluated the effect of luteal phase start ovarian stimulation on embryo euploidy rates. The current study is the first prospective study designed to evaluate embryo euploidy rates in donors undergoing two identical consecutive ovarian stimulation protocols within a period of 6 months starting either in the (FS), or (LS).

Study design, size, duration: In a prospective study, conducted between May 2018 and January 2020, 40 oocyte donors underwent two consecutive ovarian stimulation protocols within a period of 6 months with an identical fixed GnRH antagonist protocol starting either in the early follicular (FS), or and luteal menstrual cycle phase (LS).

Participants/materials, setting, methods: All participants underwent two identical consecutive ovarian stimulation cycles with 150µg corifollitropin alfa followed by 200 IU rFSH in a fixed GnRH antagonist protocol either in the FS or LS. Six MII oocytes from the same oocyte donor, from each stimulation cycle, were allocated to the recipients and were inseminated with the same sperm sample (recipients partner sperm or donor sperm). Embryos were cultivated to blastocyst stage followed by preimplantation genetic testing for aneuploidies (PGT-A).

Main results and the role of chance: When comparing FP with LP, the duration of ovarian stimulation was significantly shorter (9.68± 2.09 vs 10.93± 1.55 days), 95% CI [-1.95; -0.55] and a higher total additional dose of daily recFSH was significantly lower (526.14± 338.94 IU vs 726.14± 366.27), 95% CI [-315,12; -84,88] when CPT was administered in the luteal phase. . There were no differences in the hormone values on the triggering day (Estradiol 2137.61±1198.25 pg/ml vs 2362.96±1472.89); 95% CI [-1160.45;709.76]. Overall no differences were observed in the number of oocytes (24.84± 11.200 vs 24.27± 9.08); 95% CI[-2,61; 3.75] and MII oocytes (21.41±10.19 vs 21.59± 8.81), 95%CI [-2.72; 2.35] retrieved between FP and LP cycles in the oocytes donors. Following oocyte allocation and fertilization to the recipients, a total of 245 blastocysts were biopsied (blastocyst formation rate 245/408, 60.05%), 117 in FP group and 128 in LP group. The overall blastocyst euploidy rate was 59.18% . There were no differences in the number of euploid embryos between FS (1.59±1.32) and LS (1.70±1.29), mean difference 0.11, 95%CI [-0.65; 0.46]. Finally, there were no differences in the percentage of euploid embryos per oocytes inseminated between FS [70/287 (24.4%)] and LP [75/278 (24.7%), mean difference -0.027, 95%CI [-0.11; 0.06].

Limitations, reasons for caution: The study was performed in oocyte derived from potentially fertile young oocyte donors thus caution is needed when extrapolating the results in oocytes derived from infertile women of older age.

Wider implications of the findings: Luteal phase stimulation does not alter embryo euploidy status as compared with follicular phase stimulation and thus it appears that it can be safely used not only in cases of urgent medical fertility preservation but also in patients undergoing ovarian stimulation for IVF/ICSI.

Trial registration number: Clinical Trials Gov (NCT03555942).

P-643 Pre-selected for an award: Is Euploid blastocyst number higher in luteal versus follicular phase? A case-control study of IVF outcomes of follicular versus luteal phase ovarian stimulation

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Study question: Is there a difference between IVF outcomes in patients undergoing follicular versus luteal phase ovarian stimulation in different menstrual cycles?

Summary answer: Number of euploid blastocyst were higher in luteal phase ovarian stimulation IVF cycles. All other outcomes were similar between follicular and luteal phase IVF cycles.

What is known already: It has been published that human beings can have two or three follicular recruitment waves as observed in animals studies a long time ago. From these findings, several recent studies showed that two egg retrievals at the same menstrual cycle, named as Duo Stim, optimize time and IVF outcomes in women with low ovarian reserve due to more eggs retrieved in a shorter period with consequently higher probability of having good embryos to transfer. However, there is no knowledge about diferences concerning IVF outcomes between folicular and luteal ovarian stimulation, performed at the same women in different menstrual cycles.

Study design, size, duration: Retrospective, case-control study in a single IVF center. One-hundred-two patients who had two IVF treatments – the first cycle initiating ovarian stimulation at follicular phase (FPS) and the second cycle initiating after a spontaneous ovulation at luteal phase (LPS) – in different menstrual cycles (until 6 months apart) between 2014 and 2020, were included. Statistical analysis was performed with Mann-Whitney test and was considered significant when p≤0.05. Data is represented as mean±SD.

Participants/materials, setting, methods: Patients underwent two IVF treatments in different menstrual cycles; the FPS IVF treatment was initiating at D2/D3 of menstrual cycle and the LPS treatment started three or four days after spontaneous ovulation, if at least 4 antral follicles were detected. Both IVF treatments were performed with and antagonist protocol and freeze all strategy. The majority of patients presents low ovarian reserve/Ovarian age as primary infertility factor (84.3%).

Main results and the role of chance: Patient's mean age was 39.30±3.15 years, BMI (22.66±3.16) and AMH levels (0.85±0.85 ng/mL). Comparison of hormonal levels at the beginning of ovarian stimulation showed differences for FPS vs LPS, as expected: E2 (39.69±31,10 pg/mL vs 177.33±214.26 pg/mL,

p< 0.0001) and P4 (0.76±2.47ng/mL vs 3,00±5.00 ng/mL,p< 0.0001). However, E2 and P4 at the day of oocyte maturation trigger were not different between FPS and LPS (1355.24±895.73 pg/mL vs 1133.14±973.01 ng/mL,p=0.0883 and 1.12±1.49 ng/mL vs 2.94±6.51,p=0.0972 respectively). There was no difference for total dose of gonadotrofins (FPS 2786.43±1102.39.01UI vs LPS 2824.12±1188.87UI, p=0,8578), FSH (FPS 9.50±4.98 vs LPS 11.90±12.99, p=0.7502) and AFC (FPS 7.13±4.25 vs LPS 6.42±4.65,p=0,0944). From 102 patients that started ovarian stimulation, 78 had 1 or more oocyte collect in FPS group and 75 in LPS group: OPU (FPS 4.78±4.93 vs LPS 4.65±5.54,p=0.7889), number of MII (FPS 3.21±3.52 vs LPS 3.40±4.53,p=0.7889). From those, 52 patients performed ICSI in both cycles; fertilization rate 64.9%±28.6% for FPS vs 62.1%±32.4% for LPS,p=0.7899) and blastocyst formation 2.15±2.15 for FPS vs 2.54±2.35,p=0.3496). Data from 25 patients who had embryo biopsy for PGT-A showed similar number of blastocyst biopsed (2.12±1.72 FPS vs 2.48±1.71 LPS,p=0.3101) and a statistically significant difference regarding number of euploid blastocyst (0,20±0,41 FPS vs 0,96±0,93 LPS,p=0,0008).

Limitations, reasons for caution: This is a retrospective study in a limited number of patients. Therefore, it is not possible to make a definitive conclusion that LPS proportionate higher number of euploid than FPS. More studies are necessary to investigate not only IVF outcomes but also the impact on pregnancy rates.

Wider implications of the findings: In our study, LPS protocol after spontaneous ovulation, presents similar IVF outcomes compared to routinely FPS protocol. Intriguingly, the number of euploid blastocyst was significant higher in LPS, which may be further investigated. In this way, LPS is another option of IVF treatment, and may optimize time and treatment results.

Trial registration number: Not Applicable

P-646 Pregnancy outcomes in women with panhypopituitarism - A population-based study.

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Study question: What are the consequences of panhypopituitarism on pregnancy outcomes?

Summary answer: After controlling for confounding effects, women with panhypopituitarism have a higher prevalence of adverse obstetrical (including post-partum hemorrhage, hysterectomy and maternal death) and neonatal outcomes.

What is known already: Panhypopituitarism is a condition of inadequate or absent anterior pituitary hormone production. Pregnancy in women with panhypopituitarism is uncommon and there is only limited data (mainly case reports) regarding pregnancy outcomes in these women. Given the scarcity of data we sought to assess the association between panhypopituitarism and obstetrical and neonatal outcomes.

Study design, size, duration: A retrospective population-based study utilizing data from the Healthcare Cost and Utilization Project—Nationwide Inpatient Sample (HCUP-NIS). A dataset of all deliveries between 2004 and 2014 inclusively, was created. Within this group, all deliveries to women who had a diagnosis of panhypopituitarism during pregnancy were identified as part of the study group (n=179), and the remaining deliveries comprised the reference group (n=9,096,609).

Participants/materials, setting, methods: The HCUP-NIS is the largest inpatient sample database in the USA, and it is comprised of hospitalizations throughout the country. It provides information relating to 20% of US admissions and represents over 96% of the American population. Multivariate logistic regression analysis, controlling for confounding effects, was conducted to explore associations between panhypopituitarism and delivery and neonatal outcomes. According to Tri-Council Policy statement (2018), IRB approval was not required, given data was anonymous and publicly available.

Main results and the role of chance: Women with a diagnosis of panhypopituitarism were more likely to be older, to have a diagnosis of chronic hypertension, to have a diagnosis of pre-gestational diabetes mellitus and to be carrying twins or a higher order pregnancy (all p<0.0001), than the controls. A significantly higher risk of post-partum hemorrhage (adjusted odds ratio-aOR:3.52; 95%CI:2.18–5.69,p<0.0001), maternal infection (aOR:3.97; 95%CI:2.30–6.85,p<0.0001), pulmonary embolism (aOR:14.90; 95%CI:2.06–107.82,p<0.007), disseminated intravascular coagulation (aOR:20.29; 95%CI:10.60–38.85,p< 0.0001), maternal death (aOR:31.90; 95%CI:3.33–234.85,p=0.001) and congenital anomalies (aOR:4.55; 95CI:1.86–11.16,p=0.001), were found among the panhypopituitarism patients. Surprisingly, there was a lower incidence of caesarean delivery (aOR:0.69; 95%CI:0.50–0.96,p=0.026) in the panhypopituitarism patients than the controls. No significant difference was found in the rate of pregnancy induced hypertension (95%CI:0.78-1.97), gestational hypertension (95%CI:0.14-1.41), preeclampsia (95%CI:0.96-2.99), gestational diabetes (95%CI:0.30-1.01), preterm delivery (95%CI:0.74-1.91), preterm premature rupture of membranes (95%CI:0.17-2.82), operative vaginal delivery (95%CI: 0.23-1.19), small for gestational age neonates (95%CI:0.27-2.02) or intra-uterine fetal demise (95%CI:0.13-6.71).

Limitations, reasons for caution: The limitations of our study are its retrospective nature and the fact that it relies on an administrative database. The severity of specific hormonal deficiencies and the presence and magnitude of posterior pituitary hormone deficiencies could not be assessed, nor could compliance with hormone replacement.

Wider implications of the findings: Until now, no control studies of outcomes with panhypopituitaryism in pregnancy are available in the medical literature. Physicians should be aware of and try to prevent the above possible maternal and fetal complications related to this endocrinopathy. Future studies should evaluate the role of medication compliance with pregnancy outcomes.

Trial registration number: not applicable

P-681 Will the hCG trigger dose used for final oocyte maturation in IVF impact endogenous progesterone during the luteal phase? - A randomized controlled trial

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Study question: Is there an association between the hCG dose used for ovulation trigger and the endogenous progesterone production during the luteal phase?

Summary answer: Increased hCG dosing significantly increased the endogenous progesterone level during the luteal phase.

What is known already: During the luteal phase of an IVF treatment, the endogenous progesterone (P_4) production is negatively impacted due to reduced circulating endogenous LH, caused by negative feed-back of elevated steroids; thus, luteal phase support (LPS) with exogenous P_4 remains mandatory in IVF. Apart from inducing final oocyte maturation, the gold standard HCG trigger also functions as an early LPS, boosting P_4 production by the corpora lutea (CL). P_4 plays a pivotal role for embryo implantation and pregnancy, and an optimal P_4 level around peri-implantation seems to be essential for the reproductive outcomes of fresh and frozen/thaw embryo transfer cycles.

Study design, size, duration: A randomized controlled 4-arm study, including a total of 127 IVF patients, enrolled from January 2015 until September 2019 at the Fertility Clinic, Odense University Hospital, Denmark.

Participants/materials, setting, methods: IVF patients with ≤ 11 follicles ≥ 12 mm were randomized to four groups. Groups 1-3 were triggered with:

5.000 IU, 6.500 IU or 10.000 IU, hCG, respectively, receiving a LPS consisting of 17-α-hydroxy-progesterone (17α OH P4) to distinguish the endogenous P4 from the exogenous supplementation. Group 4 (control) was randomized to a 6.500 IU hCG trigger and standard LPS. A total of eight blood samples were drawn during the early luteal phase.

Main results and the role of chance: A total of 94 patients completed the study: 21, 22, 25 and 26 patients in each group, respectively. Baseline characteristics were similar, except for the endogenous LH level and cycle lengths. There were no significant differences between groups regarding ovarian stimulation, number of oocytes and embryos. The median number of follicles ≥ 12mm on the day of trigger was 8.5, resulting in 6.6 oocytes being retrieved. Significant differences in P_4 levels were seen at OPU+8 ($p<0.001$), OPU+10 ($p<0.001$) and OPU+14 ($p<0.001$), with positive correlations between P_4 level and hCG dose. Groups compared individually showed significant difference in P_4 between low and high trigger dose at OPU+4 group 1 and 3 ($p= 0.037$) and OPU+8 group 1 and 3 ($p=0.007$) and between all the three groups around implantation at OPU+6 group 1 and 2 ($p=0.011$), group 2 and 3 ($p=0.042$) and group 1 and 3 ($p< 0.001$). Higher P_4 levels around implantation were related to follicle count and to pregnancy. After logistic regression analyses there were still significant individual differences between the groups.

Limitations, reasons for caution: Although patients were randomized and strict inclusion and exclusion criteria were used, the RCT was un-blinded, including a relatively small number of patients. Moreover, for dosing purposes urinary hCG as well as recombinant hCG was used and pharmacokinetics differ. Finally, the P_4 level could be influenced by circadian fluctuations.

Wider implications of the findings: This is the first study to explore dose-responses in circulating P_4 after hCG trigger in IVF patients. Increasing the hCG trigger dose increased the endogenous P_4 around peri-implantation. Personalizing the hCG trigger dose could be a key point to secure the most optimal P_4 mid-luteal phase P_4 level.

Trial registration number: Eudract 2013-003304-39

POSTER DISCUSSION

SESSION 15: REPRODUCTIVE (EPI)GENETICS POSTER DISCUSSIONS

28 June 2021 Stream 3 15:15 - 16:30

P-523 Whole-chromosome aneuploidies revealed by transcriptome of trophectoderm biopsied from human pre-implantation blastocyst.

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Study question: Whether mRNA transcriptome of biopsied trophectoderm (TE) in human pre-implantation blastocyst can predict embryo karyotype?

Summary answer: mRNA transcriptome of biopsied TE can precisely predict whole-chromosome aneuploidies but not mosaicism or segmental aneuploidies.

What is known already: Karyotype of human pre-implantation blastocyst is well recognized by PGT-A. However, genome can't demonstrate gene expression level which might infer the development potential of euploidy. Transcriptome of blastocyst by singe-cell RNA-seq has revealed the lineage segregation of human pre-implantation blastocyst. It is not known whether transcriptome of biopsied TE used in PGT-A can infer the karyotype of human pre-implantation blastocyst.

Study design, size, duration: A total of 74 TE samples were biopsied from 26 blastocysts which were donated from patients who underwent PGT at our Reproductive Medicine Center. All of these embryos have been previously diagnosed as aneuploidies (n=19) or euploidies (n=7) with monogenic disorder.

Participants/materials, setting, methods: The DNA and mRNA of all biopsied TEs were separated independently using a modified oligo-dT bead capture, followed by PGT-A of DNA and smart2-sequencing of mRNA (G&T-seq). Karyotype of biopsied TEs were confirmed with PGT-A performed in MiSeq system (Illumina) in our PGT laboratory with the use of next-generation sequencing. Data of transcriptome was analyzed using Rstudio and R package InferCNV to predict aneuploidies by referring to euploidies which were inferred with corresponding PGT-A results.

Main results and the role of chance: In human pre-implantation blastocyst, all whole-chromosome aneuploidies could be inferred by transcriptome of biopsied TE, which were consistent with PGT-A result. But chromosomal mosaicism or segmental aneuploidies were hard to be predicted precisely by transcriptome of TE.

Limitations, reasons for caution: The main limitation of this study lies in the inability to retrieve the exact copy number variations from mRNA transcription. Gene expression is in a great imbalance in such an early development of human pre-implantation blastocyst.

Wider implications of the findings: Our data suggest that mRNA transcriptome is enough for prediction of whole-chromosome aneuploidies. The method and value for predicting mosaicism and segmental aneuploidies by transcriptome should be further investigated.

Trial registration number: not applicable

P-525 Analysis of segregation patterns of trivalent structure and the effect on genome stability in Robertsonian translocation carriers

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Study question: What are the factors that affect the separation pattern of Robertsonian translocation trivalent, and whether the structure of the trivalent affected the chromosome stability?

Summary answer: The meiotic segregation modes can be affected by the carrier's sex and special chromosome, and a trivalent structure can affect the stability of the genome.

What is known already: Robertson translocation occurs when two proximal acrocentric chromosomes fuse at the centromere and forms a trivalent structure during meiosis. This structure will affect the fertility of Robertsonian translocation carriers, and may destroy the stability of the genome by affecting the separation of other chromosomes, which is called Inter-Chromosomal Effect (ICE). Previous research have confirmed that the use of PGT in Robertsonian translocation carriers can effectively reduce abortion and increase live birth. But some studies dispute this conclusion and the existence of ICE. However, there is no large data study to verify these controversies.

Study design, size, duration: PGT results of 928 oocyte retrieval cycles in 763 couples(one of the couples is a Robertsonian translocation carrier) were analysied from December 2012 to June 2020. A total of 1492 couples who received PGT-A were collected as control group, and matched according to age and testing time stage. The study was approved by the ethics committee(LL-SC-SG-2006-008 and LL-SC-SG-2014-016).

Participants/materials, setting, methods: Cytogenetic analysis was performed using GTG standard method (trypsin and GiemsaG banding) to analyze the chromosomes of peripheral blood lymphocytes. Blastocysts obtained by standard IVF procedure were biopsied on the 5th or 6th morning after fertilization, and the trophoblast cells were amplified by PicoPLEX whole genome amplification kit (Rubicon Genology) or Repli-g Single Cell Kit(Qiagen). PGT-SR was performed using SNP array or NGS as previously described.

Main results and the role of chance: In this study, a total of 3423 blastocysts from 763 couples were analysed using SNP-array or NGS. Among them, the rate of alternate segregation of male Robertsonian translocation carriers was significantly higher than that in female carriers (82.26% vs 59.96%, $P < 0.001$), and meiotic segregation modes could be affected by the special chromosome

such as 13 in female(P=0.042) and 15 in male(P=0.045) involved. A trivalent structure can affect the stability of the genome during mitosis, which is associated with an increase in the propotion of chromosome mosaic compared with the PGT-A control group(1.18% vs 0.53%，P < 0.01). In addition, we found an interesting phenomenon: in the meiotic segregation of female Robertsonian translocation carriers associated with chromosomes 21 and 22, the chromosome 21 or 22 of the two chromosomes involved in translocation are more likely to be abnormal, and according to our results, the effect of chromosome 21 seems to be greater.

Limitations, reasons for caution: (1) Limitations of retrospective analysis; (2) The results are not fully representative of the general population; (3) PGT-A patients always had repeated implantation failure or recurrent aboration, which may cause deviation to the results.

Wider implications of the findings: This study analyzed the influencing factors of the separation patterns of trivalent, and verified the existence of ICE. This suggest that PGT-SR can have a better outcome in patients with Robertsonian translocation, especially in male carriers. These results will provide carrier couple with more appropriate genetic counseling.

Trial registration number: no

P-536 Pre-selected for an award: Validation of a Next Generation Sequencing (NGS) workflow integrating simultaneous analysis of ploidy, microdeletions and de novo monogenic diseases for expanded preimplantation genetic testing (PGT).

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Study question: Can major *de novo* genetic and chromosomal abnormalities (i.e., ploidy, microdeletions) be effectively tested on a single embryo biopsy specimen using an integrated NGS approach?

Summary answer: The integrated NGS workflow provided high accuracy for multilevel chromosome and genetic abnormalities analysis based on single biopsies expanding PGT informativity to *de novo* conditions.

What is known already: Current NGS-based methodologies employed in PGT for aneuploidy (PGT-A) do not detect embryo ploidy level nor frequent pathogenic *de novo* microdeletions below resolution limits. Moreover, despite their considerable incidence and adverse pregnancy outcomes, *de novo* mutations causing severe dominant monogenic fetal structural defects (FSD) are not investigated during PGT. The development of a single biopsy specimen-based PGT-A sequencing strategy that integrates ploidy and *de novo* microdeletions/mutations assessment would significantly widen PGT-A diagnostic scope and technical capabilities. This comprehensive approach would provide additional valuable genetic information of unquestionable clinical utility to further refine embryo selection process among those showing euploid profiles.

Study design, size, duration: Chromosomal conditions were validated using 24 embryo rebiopsies and 5 cell lines with both known ploidy level and known microdeletions (-4p; -8q; -1p; -22q; -5p; -15q; -11q). Genotyping for monogenic conditions was validated using 5 genomic DNA samples (33pg/µl) carrying known pathogenic Single Nucleotide Variants (SNVs) in COL1A1, SOS1, PTPN11, TSC2 and FGFR2 genes. To assess technical performance across identified SNPs, genotyping accuracy was evaluated on 17 samples from 5 embryos and 2 cell lines.

Participants/materials, setting, methods: Thirty-two *de novo* dominant monogenic conditions with FSD and strong gene-disease relationship were tested using a multiplex PCR panel with sequencing for the genes' whole coding region. Eight common microdeletions (<10Mb) syndromes (Wolf-Hirshorn, Langer-Geidion, 1p36 deletion, De George, Cri-du-Chat, Prader-Willy/Angelman, Jacobsen) were tested using B-allelic frequency (BAF) of 356 highly polymorphic Single Nucleotide Polymorphisms (SNPs). These SNPs were also used for ploidy assessment. Library preparation and sequencing were performed on the IonTorrent S5 (ThermoFisher).

Main results and the role of chance: Blinded NGS data analysis confirmed the ploidy status in all (19) samples with known constitution (8 diploids, 7 polyploids, 4 haploids). Specifically, the proportion of heterozygote calls (BAF 40%-60%) was 60.9% (95%CI:47.6-72.8) for diploid samples and <1% for haploid samples(P<0.001). All polyploid samples showed a typical splitting of BAF among 3 experimental ranges (20-40%,40%-60%,60-80%): 34.1%,18.2% and 47.7%, respectively. For microdeletions, all interstitial SNPs genotyped showed a loss of heterozygosity (LOH) as expected. The analysis of positive controls consisting of 20 blastocyst rebiopsies and 3 cell lines (-4p: n=3; -8q: n=4; -1p: n=5; -22q: n=3; -5p: n=2; -15q: n=4; -11q: n=2), allowed to accurately characterize 6 out of the 7 microdeletions (18/23 samples). In particular, all interstitial SNPs genotyped showed a LOH, while diploid controls showed an overall heterozygosity of 30.9% (average number of hetSNP x deletion=9/28). Only the very small telomeric 1p36 region failed to properly amplify. For monogenic conditions, sequencing analysis of 5 positive gDNA controls confirmed the presence of 4 known SNVs, whilst only 1 did not achieve the minimum coverage for variant calling. Moreover, 4 additional *de novo* SNVs detected by sequencing analysis in the gene panel on 8 blastocyst rebiopsies were all confirmed by qPCR/Taqman assays.

Limitations, reasons for caution: Positive controls were not available for all genes and microdeletions included in the panel. Moreover, inefficient amplification has affected some target regions and further optimization will be required. However, analytical performance on technical and biological replicates were highly promising for the tested conditions both cell lines and trophectoderm biopsies.

Wider implications of the findings: This study demonstrates that the integration of genotyping and chromosomal analyses can be efficiently achieved in the same NGS workflow. This approach can be employed to expand PGT diagnostic scope to conditions undetectable in parents due to their *de novo* onset, or that are below the standard PGT-A resolution.

Trial registration number: N/A

P-549 What trophectoderm cells from mosaic embryos tell us about embryonic competence at the transcriptional level

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Study question: Does transcriptome of remaining trophectoderm (TE) reflect the developmental potential of mosaic blastocysts after preimplantation genetic testing for aneuploidy (PGT-A)?

Summary answer: TE from low-degree mosaic (Low-mos) and high-degree mosaic (High-mos) blastocysts are transcriptionally equivalent, standing between euploid and aneuploid categories and displaying key deregulated developmental processes.

What is known already: Blastocysts classified as mosaic by PGT-A are associated with lower implantation and higher miscarriage rates than those classified as euploid, yet they still lead to healthy babies. Unveiling the true developmental identity of these embryos faces a dilemma: understanding to which extent they represent technical artefacts or whether they hold own potential to implant and give rise to normal pregnancies. Current RNA sequencing (RNA-seq) techniques allow for the determination of whole transcriptomic profiles even from single cells, which paves the way for the identification of new molecular keys of embryonic competence.

Study design, size, duration: Prospective study comparing RNA-seq data of remaining TE from blastocysts classified as euploid (n=4), Low-mos (n=5), High-mos (n=4) and aneuploid (n=6) by PGT-A. Participants were recruited between October 2018 and November 2019 at IVI-RMA Valencia.

Participants/materials, setting, methods: Chromosomal mosaicism was defined in the range 30%-<50% (Low-mos) and 50%-<70% (High-mos) using a next-generation sequencing (NGS) validated algorithm. Whole TE fractions were separately collected and processed for RNA-seq. Differentially expressed genes (DEGs) were calculated with DESeq2 package [Benjamini-Hochberg (BH)-adjusted p<0.01 & abs(log2FoldChange)>2 significant]. Fgsea algorithm was used for enrichment analysis on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms (BH-adjusted p<0.01 significant).

Main results and the role of chance: For comparisons, TE from euploid blastocysts were used as control. At the gene level, 15 DEGs were found in Low-mos, 20 DEGs in High-mos, and 64 DEGs in aneuploid blastocysts. To address the functional implications of these differences, pathways significantly deregulated according to KEGG and GO categories were identified. TE from aneuploid blastocysts displayed significant downregulation in up to 115 KEGG and GO processes directly involved in processing and integrity maintenance of nuclear and mitochondrial genomes, a reflection of their aberrant chromosomal identity. In addition, TE from High-mos and Low-mos were transcriptionally equivalent (0 DEGs between both groups), with 23 overlapping KEGG and GO processes significantly downregulated compared with control. Importantly, main significantly-affected processes included mitotic sister chromatid segregation, NIK NF-kB activity, regulation of apoptosis, and pathways related to the biosynthesis and metabolism of proteins, fatty acids, carbohydrates and steroid hormones. These findings indicate that mosaic embryos comprise a unique developmental entity, which swims between the euploid and aneuploid waterfronts and may regulate survival by diverse mechanisms, including cell proliferation and apoptosis.

Limitations, reasons for caution: This is a descriptive, single-center study with limited sample size. TE fractions were obtained by micromanipulation, which may have led to potential cross-contamination with the inner cell mass.

Wider implications of the findings: Transcriptomic equivalence between Low-mos and High-mos TE fractions questions the biological significance of inferring mosaicism degrees from single biopsies. Deregulated processes in these embryos support their reduced developmental and live birth potential, pointing to mechanisms that may mediate survival in the presence of aneuploid cells, as shown in the mouse.

Trial registration number: Not applicable

P-556 Pre-selected for an award: NAT10-mediated N4-acetylcytidine in RNA regulates mouse oocyte maturation in vitro

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Study question: Does NAT10-mediated N4-acetylcytidine (ac4C) in RNA, a newly identified mRNA epigenetic modification, participate in modulating in vitro maturation(IVM) of oocytes?

Summary answer: NAT10-mediated ac4C modification is an important regulatory factor during oocyte maturation in vitro, by regulating genes associated with translation, mitochondrial functions and protein destabilization.

What is known already: Unlike somatic cells, transcription and translation are uncoupled during oocyte maturation and gene expression is mainly regulated by post-transcriptional modulation, including mRNA degradation, translation and posttranslational modification, which are complex and have not been fully investigated. RNA ac4C is a newly identified mRNA modification and a key determinant of post-transcriptional regulation, which has been shown to promote mRNA stability and translation, and NAT10 is the only known RNA acetyltransferase. Therefore, NAT10-mediated ac4C represents a possible epigenetic regulator in oocyte maturation.

Study design, size, duration: Oocytes at different stages from mice were collected to detect the changing levels of ac4C and NAT10 during maturation. NAT10 in GV-stage oocytes was knocked down before IVM, to confirm the regulatory role of NAT10-mediated ac4C in meiotic process, followed by further exploration of cellular mechanisms. Each experiment was repeated at least three times, and data were analyzed by chi-square test, one-way ANOVA or unpaired-sample t-test.

Participants/materials, setting, methods: The expression of ac4C and NAT10 was detected by immunohistochemistry. NAT10 was knocked down in GV-stage oocytes by RNA interference through electroporation. The efficacy of knockdown was confirmed by qPCR and immunohistochemistry targeting ac4C and NAT10, and the percentages of oocytes matured in vitro were compared among groups. High-throughput sequencing and RNA immunoprecipitation were performed to reveal the modulated genes. Proteins specifically binding to ac4C sites were identified by RNA pulldown and mass spectrometry.

Main results and the role of chance: We first retrieved publicly available data from GEO and found that transcripts with potential ac4C sites were enriched in genes downregulated during IVM (P<0.001). The biased distribution of ac4C implicated a possible regulatory role. Then immunohistochemistry revealed significantly decreasing trends of ac4C and NAT10 expression from immature to mature oocytes. With NAT10 knockdown, ac4C modification was reduced and meiotic progression was significantly retarded. Specifically, the rate of first body extrusion was significantly decreased with NAT10 knockdown (34.6%) compared to control oocytes without transfection (74.6%) and oocytes transfected with control siRNA (72.6%) (p<0.001), while rates of germinal vesicle breakdown were not affected (P=0.6531). High-throughput sequencing and RNA immunoprecipitation revealed that the modulated genes were enriched in biological processes known to be associated with oocyte maturation, including translation, mitochondrial translational elongation and termination, and protein destabilization. Also, we identified a series of proteins specifically binding to ac4C probes by RNA pulldown and mass spectrometry, through which ac4C modification may exert its function in post-transcriptional modulation.

Limitations, reasons for caution: This study was performed in vitro. The role of NAT10-mediated ac4C in vivo remains to be elucidated. Also, limited by current techniques, ac4C modification in oocytes cannot be detected. Our exploration of regulated genes and ac4C binding proteins were performed in somatic cell lines.

Wider implications of the findings: Post-transcriptional modulation is crucial in oocyte maturation. Our study using in-vitro systems for mouse oocyte identified NAT10-mediated ac4C as an important regulator in IVM. It provided a new insight into the epigenetic mechanisms of IVM, which may lead to improvement of clinical IVM systems.

Trial registration number: not applicable

POSTER DISCUSSION

SESSION 16: PSYCHOLOGY AND COUNSELLING POSTER DISCUSSIONS

28 June 2021 Stream 4 15:15 - 16:30

P-479 Are FET and IUI cycles less emotionally difficult for patients than IVF? Evidence from smartphone app based emotional tracking data

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Study question: Is the emotional experience different in FET and stimulated IUI cycles compared to IVF cycles?

Summary answer: Emotional tracking data demonstrated cautious optimism and lower harm emotions in IUI, but FET cycles are associated with higher harm emotions than fresh IVF.

What is known already: It is sometimes claimed on clinic websites and by advocates for elective freeze all that FET cycles are inherently less stressful. However, little research evaluates the emotional difference between fresh and frozen cycles and the assumed emotional ease of FET may reflect clinician interpretation/bias rather than patient's lived experiences. Many undertaking FET will have experienced disappointment in a fresh cycle and with increasing cycles comes increased cost.

IUI treatment is perceived as less physically and emotionally intense, but studies have shown increased depression levels after a failed IUI cycle and high drop-out.

Study design, size, duration: Retrospective single-centre analysis of anonymised emotional tracking data entered by 707 patients using MediEmo app alongside IVF, 104 during stimulated IUI and 65 during medicated FET from May 2017-September 2020.

MediEmo includes medication timeline/ notifications, coping tools and emotional tracking. Patients rate 2 questions daily in each emotion domain (challenge, threat, harm, e.g. 'I am feeling tense') on a 0-3 scale and indicate coping ability ('I am unable to cope with the stress I'm experiencing').

Participants/materials, setting, methods: Egg donor, recipient and fertility preservation cycles were excluded. Mean and standard deviation of scores in each mood domain entered per cycle day were calculated, centred on luteal day 0/ egg collection, from cycle day +/-14.

Between group analysis performed using one-way analysis of variance (ANOVA) is presented here. Time series analysis, graphical presentation of emotions by cycle day and analysis of cycles resulting in live birth or return for further treatment will be presented.

Main results and the role of chance: Analysis of emotional tracking data demonstrated patients experience higher levels of positive challenge emotions (confident/encouraged/hopeful/positive) during FET and IUI cycles than fresh IVF: mean(s.d) score FET 1.64(1.1), IUI 1.74(0.89), IVF 1.48(1.06) (ANOVA $p<0.00001$). The difference between FET and IUI challenge levels was not significant ($p=0.07$).

Threat emotions (worried/nervous/anxious/tense) are significantly lower in FET compared to IVF and IUI cycles: FET mean 0.67(0.91), IUI 0.97(0.90), IVF 0.87(0.91), (ANOVA $p<0.00001$). The difference between IVF and IUI threat levels was not significant ($p=0.06$).

However, the harm emotions (sad/discouraged/disappointed) experienced by patients are significantly higher in FET, mean 0.62(0.89) compared to IVF 0.50(0.81), which are higher than IUI cycles, 0.36(0.68), (ANOVA $p<0.00001$). There were no significant differences in numbers recording intolerable stress between the three groups (FET mean scores 0.24(0.66), IUI 0.21(0.58), IVF 0.21(0.59), (ANOVA $p=0.67$).

As this is retrospective observational data, there are differences between groups in addition to treatment modality, e.g. mean patient ages in the FET and IUI groups were older than those entering data during IVF; FET 34.2(4.09), IUI 33.9(5.2), IVF 32.6(4.47). However, age was not correlated with levels of challenge emotions, suggesting assumptions that patient emotions, e.g. hopefulness, are closely linked to objective prognosis may be flawed.

Limitations, reasons for caution: Emotional data was only available for those who chose to use MediEmo, entered emotional tracking data and who gave consent for use of data in research. As such, this analysis may not fully reflect all patients' experiences. However, these limitations apply to all groups and should not prevent useful comparison.

Wider implications of the findings: Patients have less contact with clinic staff during FET or IUI than fresh IVF cycles. Fertility staff need to ensure availability of support during all treatment cycles and be empathic, particularly for those embarking on FET, who may still be coming to terms with a failed fresh transfer cycle.

Trial registration number: Not applicable

P-490 Recurrent pregnancy loss acts as a posttraumatic stress event in both women and men

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[2]University Hospital, Institute of Medical Psychology- Center for Psychosocial Medicine, Heidelberg, Germany

Study question: What are the psychological impacts of recurrent pregnancy loss (RPL) on men and women and their interdependencies?

Summary answer: Women show higher psychological risks than men, except for lack of social support. Avoidance behaviour of men correlates with higher posttraumatic stress of their partner.

What is known already: About 1-3% of all couples trying to conceive are affected by RPL. The loss of the unborn child can be the most traumatic experience in a woman's life and is associated with significant psychological distress besides the instant grief. RPL can also be stressful for the partner, even though being at a lower risk for psychiatric morbidities. The man's gender role expects him to support and not to show weakness which may result in a suppression of his feelings and a disenfranchised grief.

Study design, size, duration: Cross-sectional study. All women and men referred to the special unit for RPL between March 2019 and October 2020 were asked to participate voluntarily with a total sample size of 105 couples and 17 women. Exclusion criteria were less than two pregnancy losses, inadequate knowledge of the German language and incomplete data.

Participants/materials, setting, methods: Couples were invited to fill out a questionnaire package estimating their psychological risks (e.g. posttraumatic stress disorder, anxiety, depression, perceived social support) and coping strategies with established instruments. Clinical history was obtained from medical records. Couple data were analysed with the Actor Partner Interdependence Model, taking the couple as the unit of analysis.

Main results and the role of chance: The response rate was 82.3%. The following psychological risks were measured among women versus men: posttraumatic stress disorder (PTSD): 13.7% versus 3.9% ($p=0.017$); anxiety: 50.4% versus 17.3% ($p<0.001$), depression: 48.1% versus 14.4% ($p<0.001$), lack of social support: 32.5% versus 32.7% (N.S.). A risk in at least one category showed 68.9% of women versus 44.8% of men ($p<0.001$), with those at higher risk indicating a lower satisfaction with their partnership ($p<0.001$) and higher impairment of their sexual life ($p<0.001$). Neither total number of pregnancy losses nor time gap since last pregnancy loss showed relevant correlations with psychological risks. For women, number of curettages, controlled for the number of pregnancy losses, correlates with the severity of posttraumatic stress ($p<0.05$). Higher levels of anxiety, depression and a lack of social support in women correlated positively with posttraumatic stress in their partners. The coping strategy "trivialization and wishful thinking" as well as the subscale "avoidance" of the Impact-of-Event-Scale (self-report questionnaire measuring posttraumatic stress) of men was correlated with more severe posttraumatic stress in their female partners (both $p<0.05$).

Limitations, reasons for caution: The data show only correlations between the measured variables, as cross-sectional studies are not suitable to analyse causal relationships. The sample was obtained in a special unit for RPL at a university hospital, so the findings may not be generalizable to all couples with RPL.

Wider implications of the findings: Screening psychological risks in couples with RPL may be reasonable considering the high risks in both sexes and the extent of PTSD diagnoses in women, their interdependencies and the potential risk of chronification. Professionals should encourage affected couples to support each other and provide targeted information on mental health services.

Trial registration number: DRKS 00014965

P-503 Focus groups with health care professionals, patient advocates and patients to explore how the potential need for multiple cycles is managed during fertility treatment consultations

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[1]Psychology, School of Psychology- Cardiff University, Cardiff, United Kingdom

Study question: How is possibility of failure and potential need for multiple cycles discussed with patients during the first or repeat IVF/ICSI treatment consultation?

Summary answer: Health Care Professionals plan treatment on a cycle-by-cycle basis because it is the normative way to plan treatment, but patients see advantages in multi-cycle planning

What is known already: Many patients need more than one round of IVF/ICSI stimulation to achieve their parenthood goals. A recent study has found around 60% of patients to be willing to plan for multiple cycles of treatment. However, it is not clear how patients are informed fully about the high possibility of treatment failure and the subsequent need for multiple cycles during their treatment planning consultations

Study design, size, duration: Qualitative focus groups with health care professionals (HCP) patient advocates (April 2020) and patients (July and August 2020, respectively). Patients were eligible if they had had a consultation to start a first/repeat stimulated IVF/ICSI cycle in the eight weeks prior to participation, were aged 18 or older (upper age limit of 42 years for women) and fluent in English. Eligible Health HCPs and patient advocates were those employed at a fertility clinic or charity, respectively

Participants/materials, setting, methods: HCP, patient advocate and patient focus group topic guides started with general questions about fertility consultations and progressed to discuss if and how the possibility of treatment failure and need for multiple cycles was introduced and discussed, and then preferences regarding planning IVF/ICSI on a multi-cycle rather than a single cycle basis. Focus groups were recorded, transcribed and analysed using framework analysis which allowed examination of shared, unique and incongruent thematic content across participant groups

Main results and the role of chance: Twelve HCPs, 2 patient advocates and 11 patients participated in seven semi-structured online focus group discussions. Framework analysis revealed 52 codes (e.g., possibility of failure tentatively introduced; discussion of multiple cycles dependent on clinical/patient benchmarks) abstracted into 17 higher-level categories (e.g., Failure is a sensitive topic to approach; IVF treatment failure is the norm). Synthesis of categories revealed four themes and one meta theme The meta theme showed planning treatment on a cycle-by-cycle basis was the norm. This meta-theme was supported by four themes: (1) 'culture of communication' that dictated benchmarks (e.g., clinic, national live birth rate) and definition of key concepts ('complete' cycle) that underpinned divergence between clinics; (2) 'HCP-patient dynamics' indexing degree of shared decision-making, advance preparation and involvement of partners in planning; (3) 'tempering optimism' that described tailoring, balancing and emotion management in giving personal chances of success; and (4) 'transitioning to multi-cycle planning' which identified worries of multi-cycle planning (e.g., need to learn from failure).

Limitations, reasons for caution: The majority of patients were women from private fertility clinics with no previous treatment experience recruited from social media websites, mainly associated with patient support groups. Similarly, the majority of HCPs were women from private fertility clinics. Informative comparisons across treatment stage, gender and funding source were therefore not possible

Wider implications of the findings: HCPs are hesitant towards multi-cycle planning. However, patients show openness, suggesting a cultural shift from the single cycle norm of planning IVF/ICSI may be possible. If adopted by clinics, HCPs patients and fertility organisations, multi-cycle planning could encourage patients to create informed treatment expectations and plans prior to treatment engagement

Trial registration number: MS200059_001

P-504 A randomised controlled trial comparing expectant management or intrauterine-insemination in couples with unexplained subfertility and a poor prognosis for natural conception: the impact on health-related-quality-of-life

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[4]Jeroen Bosch Hospital, Obstetrics and Gynaecology, Den Bosch, The Netherlands ;
[5]Leiden University Medical Centre, Obstetrics and Gynaecology, Leiden, The Netherlands ;
[6]University Medical Centre Groningen, Obstetrics and Gynaecology, Groningen, The Netherlands

Study question: Is health-related quality of life (HRQoL) in women with unexplained subfertility and a poor prognosis influenced by expectant management or intrauterine insemination with ovarian stimulation?

Summary answer: HRQoL did not differ, except for the relational domain which was lower after expectant management. Anxiety and depression disorders occurred frequently in both groups.

What is known already: In couples with unexplained subfertility and a poor prognosis, IUI with ovarian stimulation (IUI-OS) is a first line treatment. Not much is known about quality of live or depression and anxiety in these couples. The Fertility Quality of Life (FertiQoL) is reliable for assessment within relational and social domains, the Hospital Anxiety and Depression Scale (HADS) is a reliable tool to detect anxiety and depression disorders.

Study design, size, duration: We performed a multicentre RCT in couples with unexplained subfertility with a poor prognosis of conceiving naturally within one year. Women were allocated 1:1 to six months expectant management or to six months IUI-OS. HRQoL was assessed with standard self-administered psychometric measures with established reliability and validity: FertiQol and HADS. We intended to include 1091 couples but after almost 4 years, the study had to stop due to slow inclusion and therefore lack of funding.

Participants/materials, setting, methods: Between June 2017 and September 2020, we recruited 178 women of wich 92 were assigned expectant management and 86 IUI-OS. All women who participated and could read Dutch were eligible for the HRQoL measurements because HRQoL questionnaires in foreign languages were not yet available online. Women completed the questionnaires before randomisation, 3 and 6 months after randomisation. We used mixed model analyses to assess differences between treatment groups and the effect of time.

Main results and the role of chance: One hundred sixty-two women could read Dutch and were invited (162/178 (91%)). Analyzable data of the FertiQol questionnaire were available for 80% (130/162). Compared to women allocated to IUI-OS, women allocated to expectant management had a lower FertiQol score in the relational domain (mean difference -4.3 (95% CI -7.3 to -1.3) but not in the social domain (mean diff van -0.8 (95% CI -4.5 to 2.9).

Data of the HADS questionnaire were available of 156 women (96% (156/162)). Both groups had comparable scores in the Anxiety (mean difference -0.20; 95% CI 0.63; -0.99 to 0.6) and Depressions score (mean difference 0.002; 95% CI -0.67 to 0.67) at all three moments. At baseline, the incidence of an anxiety disorder (definition score 8 or higher) was 19% (30/156) and increased to 30% and 29% at 3 months and 6 months respectively. The incidence of a depression disorder (definition score 8 or higher) was 5% (7/156) and increased to 16% and 18% at 3 months and 6 months respectively. The incidences of anxiety or depression disorders did not differ significantly between expectant management and IUI.

Limitations, reasons for caution: Our randomized controlled trial did not reach the planned sample size. The results are only applicable to women with unexplained subfertility and a poor prognosis and not to all women with unexplained subfertility.

Wider implications of the findings: Although often assumed, IUI-OS does not improve HRQoL compared to expectant management in all domains. IUI might prevent loss of quality of the relationship, but the impact seems small. Future studies should look into the high incidence of anxiety and depression disorders in these women and how to support them.

Trial registration number: Trial register NL5455 (NTR5599)

P-513 Analysis of the extent of dropout-rates by extraction from cumulative live birth rates in IVF/ICSI: systematic review and meta-analysis

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Study question: The purpose of this systematic review is to calculate dropout-rates of IVF/ICSI treatment by analysing the published cumulative live birth rates of IVF/ICSI treatment.

Summary answer: One out of three patients stop their treatment after their first IVF/ICSI cycle and dropout-rates tend to increase per consecutive cycle.

What is known already: Cumulative live birth rates (CLBRs) have created the possibility to present realistic probabilities of having a live birth after IVF/ICSI treatment. However, it is noted that a significant percentage of the patients stop their treatment before having a child ("dropout"). Possible reasons and predicting factors for dropout of treatment are already extensively investigated. However, only a few studies try to report about the incidence of dropout. Publications on CLBRs of large numbers of patients allow the extraction of dropout-rates. These rates will provide insight in the extent of the problem and could be used as a reference for interventional studies.

Study design, size, duration: Four databases (PubMed, The Cochrane Library, EMBASE, DoKS) were systematically searched from 1992 to December 2020. Search terms referred to "cumulative live birth" AND "ART/IVF/ICSI". No restrictions were made on the type or language of publication. Studies were included if they reported absolute numbers of patients and live births per

consecutive complete IVF/ICSI cycle or per consecutive embryo transfer cycle, starting from the first IVF/ICSI cycle for each patient.

Participants/materials, setting, methods: Dropout-rates per cycle were calculated in two manners: "intrinsic dropout-rate" with all patients that started the particular IVF/ICSI cycle in the denominator, and "potential dropout-rate" with all patients who did not achieve a live birth after IVF/ICSI (and potentially could have started a consecutive cycle) in the denominator. Dropout-rates were analysed for consecutive complete cycles and consecutive embryo transfer cycles, because these two manners are used in reporting CLBRs, often related to the reimbursement policy.

Main results and the role of chance: This review included 29 studies and almost 800,000 patients from different countries and registries.

Regarding the patients who started their first IVF/ICSI cycle, trying to conceive their first child by IVF/ICSI, intrinsic dropout-rate was 33% (weighted average) after the first complete cycle, meaning they did not return for their second oocyte retrieval cycle. After the first embryo transfer cycle, intrinsic dropout-rate was 27% (weighted average), meaning those patients did not return for their next frozen-thawed embryo transfer cycle or for the next oocyte retrieval cycle. Regarding the patients who did not achieve a live birth after the first complete cycle, potential dropout-rate was 48% (weighted average), and 37% (weighted average) after the first embryo transfer cycle.

Both potential and intrinsic dropout-rates for both consecutive complete and embryo transfer cycles tended to increase with cycle number.

One study on second IVF/ICSI conceived children showed a potential dropout-rate after the first complete cycle of 29%. From studies on women >40 years of age, the potential dropout-rate after the first complete cycle was 45% (weighted average) and from studies with the uses of testicular sperm extraction, the potential dropout-rate after the first complete cycle was 34% (weighted average).

Limitations, reasons for caution: Our analysis was hampered by the different ways of reporting on CLBRs (complete cycles versus embryo transfer cycles), informative censoring, patients changing clinics and spontaneous pregnancies. Dropout-rates were potentially overestimated given that spontaneous pregnancies were not taken into account.

Wider implications of the findings: The extent of dropout in IVF/ICSI treatment is substantial and has an important impact on its effectiveness. Therefore, it is a challenge for fertility centers to try to keep patients longer on board, by taking into account the patients' preferences and managing their expectations.

Trial registration number: PROSPERO Registration number: CRD42020223512

INVITED SESSION

SESSION 17: THE OOCYTE - THE LEADING LADY IN FEMALE FERTILITY PRESERVATION

28 June 2021 Stream 1 17:00 - 18:00

O-014 How effective is oocyte cryopreservation?

A. Cobo[1]

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Abstract text

The challenge of cryopreserve, store for prolonged period, and successfully implant the female gamete is nowadays feasible thanks to vitrification. The technology that was initially validated in oocyte recipients is currently applied to a vast population, including women at risk of losing their ovarian function due either to iatrogenic causes as occurs in cancer patients, or due to the natural depletion of the ovarian reserve as a result of age related fertility decline. That is the case of a growing population of women who wish to postpone childbearing and decide on oocyte vitrification as a means of fertility preservation (FP). At present, there is a growing body of evidence regarding the use of vitrified oocytes by many women under different indications, which makes it possible to evaluate the approach from different scenarios. So that vitrification can be evaluated in terms on survival rates, embryo development and the rate at which vitrified oocytes develop into live-born children in IVF cycles using vitrified oocytes which were initially stored due to different reasons. The effects of vitrification at the subcellular level and its impact on oocyte competence is of interest in the evaluation of the efficacy of the technology. Some studies have indicated that vitrification may affect ultrastructure, reactive oxygen species (ROS) generation, gene expression, and epigenetic status. However, it is still controversial whether oocyte vitrification could induce DNA damage in the oocytes and the resulting early embryos. Recent studies show that oocytes survival and clinical outcome after vitrification can be impaired by patients' age and the clinical indication or the reason for vitrification. These studies show that age at oocyte retrieval strongly affects the survival and reproductive prognosis. In our experience, oocyte survival, pregnancy and cumulative live birth rates are significantly higher when patients are aged 35 years or younger versus patients older than 35 years at oocyte retrieval. Therefore, elective-FP patients should be encouraged to decide at young ages to significantly increase their chances of success. There is also evidence that the reason for vitrification is associated to the success rates. Poorer reproductive outcome was reported in cancer patients, low responders and endometriosis patients when compared to healthy women in age matching groups. Moreover, there are certain individualities linked to specific populations, as occurs when endometriosis patients had cystectomy earlier than the oocyte retrieval for FP. These women achieved lower success rates as compared to non-operated age matching counterparts. In this case, the lower cumulative live birth rates observed in operated women are, most probably, due to the smaller number of oocytes available, as a consequence of the detrimental effect of the surgery on the ovarian reserve. In this regard, several reports show that the number of oocytes available per patient is another variable closely related to the outcome in all populations using vitrified oocytes after FP. Thus, a significant improvement in the cumulative live birth rates can be achieved by adding a few oocytes, especially in healthy young patients. Different populations using vitrified oocytes under several indications achieve differential results in terms of pregnancy rates, when calculated in overall. Nonetheless, when the calculations for the cumulative probability of achieving a baby are made according the number of oocytes used per patient belonging to the same group of age, the results become comparable between different populations, as shown by the comparison between elective freezers versus endometriosis patients. Undoubtedly, vitrification can be recognized as one of the latest brakethrough in the ART field, but certainly the next step forward would be the successfull automatization of the vitrification and warming processes to achieve fully consistency among different laboratories.

O-015 How do genes impact?

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INVITED SESSION

SESSION 18: PATIENT EMPOWERMENT DURING MAR PROCEDURES

28 June 2021 Stream 2 17:00 - 18:00

O-016 Hypnofertility for reducing stress and increasing fertility preparedness

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Abstract text

Using systematic, holistic and knowledge-based approaches for support the couples by nurses may be effective in reducing stress and increasing pregnancy outcomes, Hypnofertility is one of the methods that can help in reducing stress and increase fertility preparedness of couples. This program was first formed in 2013 by the HypnoBirthing Institute trainer, Sherry Gilbert. Its basic principle is that fertility is a natural function. The program is based on powerful and effective mind-body interaction. Mind consciously or unconsciously records all of our experiences and external messages. The purpose of this presentation is to present Hypnofertility method and to provide a systematic approach to reduce the stress of couples who receive fertility support. The mind functionally consists of

consciousness, subconscious and critical factors. Consciousness is the part that analyses, makes logic, and where reality takes place. It records short-term memory. In consciousness; personal boundaries, attitudes, beliefs, decisions, hope for the future, and thoughts. For people with fertility problems words such as infertile, unsuccessful, sterile, complete failure, complex, difficult, dysfunction, disorder, damage, deficiency, too late they hear often. The experiences and these negative messages can lead to the belief that the treatment and the person will fail; make the decision to stop treatment; despair about pregnancy; can lead to difficult, complex thoughts. The subconscious is the creative, emotional part that accepts without separating right or wrong like the computer, does not think rationally. It records long-term memory. It stores all positive/negative experiences from intrauterine life to adulthood. In our subconscious are emotions, behaviours, experiences and way of thinking. Emotions such as inefficient, inadequate, unhappy are often found in the subconscious of couples who live fertility problems. They adopt this way of thinking and experience stress as a behaviour. Finally, the critical factor is located between consciousness and subconscious. Our conscious state does not accept messages without judgment. On the contrary, our subconscious accepts all messages, positive or negative. Hypnofertility improves critical factor barrier. It activates and prevents negative messages from settling in the subconscious while allowing positive messages to be settled. How the consciousness can be affected? The language of consciousness are words. Affirmations are used to positively affect consciousness of couples. The affirmations should be personal, positive, precise, present, sensible and applicable. For example: Instead of saying "Your eggs are undeveloped, few and small", "Your eggs continue to develop, we need a little more time to collect them." The subconscious can be affected visualization, relaxation, and imagination. Creating a visual in the mind of a person and adding taste, smell and sound to it. For example; hanging pictures of couples who have children as a result of treatment in the clinic can help the couples to create the picture in their minds. In addition, the imagination technique allows the individual to mentally move away from the uncomfortable environment, relieve tension, and provide mental and physical relaxation. For example; by imagining blood flow to the uterus during and after embryo transfer, the person can activate the parasympathetic nervous system and relax, so can improve blood flow to the uterus. Relaxation is the relief of the person's body by breathing, visualization, mental space building and music can be used during it. Hypnofertility support program is non-invasive, cheap and easy to apply.. This program of care is a systematic, holistic and knowledge-based and it can reduce the stress, improve fertility preparedness, increase pregnancy outcomes, and increase the trust and confidence in the nurse. It is thought to be effective in changing perspective. Researcher nurses also can plan studies in order to evaluate its effectiveness on stress and pregnancy outcomes.

O-017 Appeased embryo transfer with hypnosis

D. Lelaidier[1]

[1]*centre PMA saint ROCH, hypnosis IVF center st Roch, Montpellier, France*

Abstract text

Objective,

We report in this presentation the use for couples undergoing infertility treatment of a new way of accompaniment. Hypnosis associated with learning of self-hypnosis is a solid support to valid an appeased uptake of an infertility program and lower the emotional charge associated with such treatments.

Main: enhance emotional comfort in couples undergoing infertility treatment.

Secondary: patients feelings after results of the attempt (whether failure or success), pregnancy rates.

Contains

During a first meeting family and historical back ground is analyzed as well as medical file and causes for infertility. Then a first specific session is proposed in relation with underlying problems using ericksonian hypnosis. For example sessions using amnesia can be used in patients having had traumatic experiences. Comfort and wellbeing suggestions are used after each hypnosis session.

Two other sessions can be proposed at office, one called "the two chests" first one to pack all past failures and second to collect present or past successes regarding all fields. These successes will be resourceful to refer to. The second session will be to enhance self-confidence using contes.

In order to enhance autonomy patients are given 4 audio sessions prerecorded to home practice.

Three of them are specific to intra uterine insemination or embryo transfer. One is called FIVETE to listen the day before medical procedure, one is called SIMPLE INDUCTION to start just before and throughout the procedure. One to do after procedure at home called DO NOTHING.

Patients are called few weeks after the attempt for debrief and results.

INVITED SESSION

SESSION 19: EXCHANGE SESSION- ASRM: COVID AND ENDOMETRIOSIS: TWO INFLAMMATORY DISEASES WITH MULTI-ORGAN EFFECTS.

28 June 2021 Stream 3 17:00 - 18:00

O-018 Endometriosis is a Systemic disease

H. Taylor[1]

[1]*Yale School of Medicine, Dept. of Reproductive Endocrinology and Infertility, New Haven, CT, U.S.A*

O-019 COVID – Impact on reproduction and reproductive practice

M. Cedars[1]

[1]*UCSF, Obstetrics- Gynecology and Reproductive Sciences, San Francisco- CA, U.S.A.*

Abstract text

A highly infectious novel coronavirus (now referred to as SARS-CoV-2) was first noted in December 2019 in Wuhan, Hubei Province, China, and by March 11, 2020, was declared a global pandemic by the WHO. The widespread community transmission of a virus, new to our species, continues to raise urgent questions about implications for pregnant women and those considering conception. Almost immediately, international committees, including ASRM and ESHRE, drew up guidelines to protect the public and our patients. Across the globe, clinics were closed, patients turned away and questions regarding spread of the virus, safety during early pregnancy and potential impact on fertility and pregnancy began to arise. Where are we now? What have we learned? And what more do we need to know to improve our ability to care for and counsel our patients?

Clinic Practice – While there was considerable controversy in the U.S., closing clinics was the correct course of action when an unknown virus had entered our countries and so little was known and resources (think NY, think Italy) were inadequate. The majority of clinics pivoted to more virtual visits and stopped transfers and retrievals. The duration of these changes varied across states and countries, with most clinics now functioning at full capacity for procedures but still utilizing virtual visits for many patients. We will discuss what we learned from this process, including impact on clinics and patients, as well as the greater community in which we all live.

Pregnancy – The physiology of pregnancy, including increased heart rate and oxygen consumption, decreased lung capacity and a shift away from cell-mediated immunity, all increased the risk for severe illness. Studies have now shown this increased risk for severe disease, mechanical ventilation and even death in pregnant women compared with their non-pregnant counterparts. Additionally, there is increased risk for pre-term labor and fetal death. Studies suggest infection earlier in pregnancy increases risk for complications. What about our patients? The first trimester is a highly critical period for fetal development. As a result, infectious and non-infectious exposures, occurring during the first trimester, are most likely to lead to severe effects on fetal development. Preliminary results no increased risk for pregnancy loss and no effect on nuchal translucency. However, some studies have shown the possibility of vertical transmission and increase in fetal inflammation. We will review the literature and update on current understanding of first trimester exposure and consequences for both mother and child.

Infertility – SARS-CoV-2 utilizes the angiotensin-converting enzyme 2 (ACE2) receptor for viral entry. The ACE2 receptor is present in both the male and female reproductive systems. Early case studies of severe cases of COVID-19

identified orchitis, while the presence in non-fatal disease remains controversial. Lowered sperm counts have been identified and some studies have found SARS-CoV-2 viral particles in the semen. ACE2 receptor is present in both the ovary and the endometrium, while infection is possible, there have been few studies specifically looking at these endpoints and no clear risk identified for women.

Vaccination – The rapid development and deployment of effective vaccination has brought hope to end the pandemic, even as new variants are arising. While vaccine hesitancy is common in many places, the mis-information regarding association between vaccines and infertility has hit our field particularly hard. Updating information to share with our patients, colleagues and friends will be critical to move forward and combat the pandemic.

Trial registration number:
Study funding:
Funding source:

SELECTED ORAL COMMUNICATIONS
SESSION 20: NOVEL TECHNOLOGIES IN REPRODUCTION

28 June 2021 Stream 4 17:00 - 18:00

O-099 TEAD4 regulates trophectoderm differentiation upstream of CDX2 in human preimplantation embryos

P. Stamatiadis[1], A. Boel[1], G. Cosemans[1], F. Van Nieuwerburgh[2], B. Menten[3], P. De Sutter[1], D. Stoop[1], S. M. Chuva de Sousa Lopes[4], F. Lluis[5]

[1]Ghent University, Department for Reproductive Medicine Ghent University Hospital, Gent, Belgium ;
[2]Ghent University, Laboratory of Pharmaceutical Biotechnology, Gent, Belgium ;
[3]Ghent University, Center for Medical Genetics- Department of Biomolecular Medicine, Gent, Belgium ;
[4]Leiden University Medical Centre, Department of Anatomy and Embryology, Leiden, The Netherlands ;
[5]KU Leuven, Department of Development and Regeneration, Leuven, Belgium

Study question: What is the main pathway regulating trophectoderm (TE) differentiation during pre-implantation development in mouse versus human embryos?

Summary answer: *TEAD4* is acting upstream of *CDX2* and is involved in TE differentiation, as TEAD4-null human embryos exhibit compromised TE lineage differentiation.

What is known already: TEAD4 is the earliest transcription factor during early embryo development, required for the expression of TE-associated genes leading to successful TE differentiation and subsequent blastocoel formation in mouse. Functional knock-out studies in mouse, inactivating *Tead4* by site-specific recombination have shown that *Tead4*-null embryos do not express TE specific genes, including Caudal-Type Homeobox Protein 2 (*Cdx2*) and GATA Binding Protein 3 (*Gata3*), but expression of inner cell mass (ICM)-specific genes, remains unaffected. Furthermore, ablation of *Tead4* compromises embryonic development and subsequent blastocoel formation in mouse. The role of *TEAD4*, during human pre-implantation development has not been functionally characterized yet.

Study design, size, duration: CRISPR-Cas9 was introduced in mouse zygotes and editing efficiency was evaluated by next-generation sequencing (NGS) on 4.5dpc embryos (n=55). Developmental kinetics were monitored in CRISPR-Cas9 targeted (n=83), sham-injected (n=26) and non-injected media-control (n=51) mouse embryos. Immunofluorescence analysis was performed in *Tead4* targeted (n=57) and non-injected media-control embryos (n=94). The same methodology was applied in human donated *in vitro* matured (IVM) metaphase-II (MII) oocytes, which were CRISPR-Cas9 targeted (n=74) during ICSI or used as media-Control (n=33).

Participants/materials, setting, methods: A gRNA-Cas9 mixture targeting exon 2 of *Tead4/TEAD4* was microinjected in respectively mouse 2PN (pronuclear) stage zygotes, or human IVM MII oocytes along with the sperm. Generated embryos were cultured *in vitro* for 4 days in mouse or 6.5 days in human. Embryonic development and morphology were assessed daily, followed by a detailed scoring at the late blastocyst stage. Successful targeting following CRISPR-Cas9 introduction was assessed by immunostaining and NGS analysis of the targeted locus.

Main results and the role of chance: In mouse, we confirmed previous findings, as the developmental capacity of *Tead4* targeted embryos was significantly reduced starting from the morula stage and blastocyst formation rates were 8.97% in the targeted group, compared to 87.23% in the control and 87.50% in the sham group, respectively. Immunofluorescence analysis of late morula and blastocyst stage embryos confirmed the absence of Tead4, Cdx2 and Gata3, resulting from the successful interruption of the *Tead4* locus (n=57). Exon 2 of *TEAD4* was successfully targeted in human. In total, 21 embryos from various developmental stages were successfully NGS analyzed and 90,48% (19 out of 21) of the embryos carried genetic modifications as a result of CRISPR-Cas9 genome editing and seven blastocysts were identified carrying exclusively frameshift mutations. In contrast to mouse, the developmental capacity of human targeted embryos (25%) did not differ significantly from the control group (23%). However, the blastocyst morphology and quality were compromised in the targeted group showing mostly grade C TE scores, containing very few cells. Immunofluorescence analysis of targeted blastocysts (n=6) confirmed successful editing by complete absence of TEAD4 and its downstream TE marker CDX2.

Limitations, reasons for caution: CRISPR-Cas9 germline genome editing results in multiple editing outcomes with variable phenotypic penetrance, the mosaic nature of which complicates the phenotypic analysis and developmental behaviour of the injected embryos.

Wider implications of the findings: Elucidation of the evolutionary conserved molecular mechanisms that regulate self-renewal of the trophoblast lineage can give us fundamental insights on early implantation failure.

Trial registration number: Not Applicable

O-100 The follicular fluid derived exosomes facilitate the differentiation of oocytes from human induced pluripotent stem cells through miR218 / PI3K / Akt molecular axis

Abstract withdrawn by the authors

O-101 Neospermatogenesis benefits from a three-dimensional culture system

S. Lawrence[1], M. Haddad[1], Z. Rosenwaks[1], G.D. Palermo[1]

[1]Weill Cornell Medicine, The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, New York, U.S.A.

Study question: Does a three-dimensional (3D) culture system increase the efficiency of male germline differentiation of mouse embryonic stem cells (mESC) over a bidimensional method?

Summary answer: Our 3D culture system based on direct spherification proves superior to the standard bidimensional plating in promoting neogametogenesis of mESC into post-meiotic male germ cells.

What is known already: Two-dimensional monolayer cell cultures are common in stem cell research. However, this method does not replicate a physiological 3D spatial relationship and may provide an inaccurate replication of *in vivo* environments. A 3D spherical structure allows us to mimic the seminiferous tubule, the site of *in vivo* spermatogenesis. By using spheroids as a scaffold to replicate cell culture systems, we can study spermatogenesis in a controlled setting. Direct spherification, a technique commonly used in molecular gastronomy, provides an opportunity to create spheroids that mimic *in vivo* events that materialize in the lab

Study design, size, duration: mESCs were initially cultured on a 6-well plate coated with fibroblasts and inserted into sodium alginate spheres. To coax differentiation, spheres (3 to 6 mm in diameter) were plunged directly into differentiation medium (DM) while the control mESC in 6-well dishes were layered with it. Cells obtained from both culture systems were tested by biomarkers for different germ cell stages

Participants/materials, setting, methods: Bidimensional mESC at 80% confluence were differentiated either on a plate or spherified for a 3D culture. Both systems underwent the same timeline of exposure to EpiLC medium with Activin A, bFGF and KSR for 3 days and PGCLC medium with BMP4, LIF, SCF and EGF for 7 days. Differentiated cells were retrieved from each method at day 3 and day 10 to assess for germ line differentiation markers, DAZL, VASA and BOULE

Main results and the role of chance: Under optic visualization through the sphere wall, cellular aggregation was seen on day 2 of culturing in EpiLC medium while this phenomenon was not observed on bidimensional plating. In the conventional method, cells expressed 7% DAZL (spermatogonium cell stage) and 1% VASA (pre-spermatid cell stage) whereas in direct spherification, cells expressed 20% DAZL ($P<0.001$) and 15% VASA positivity ($P<0.0001$).

To further compare the different methods in later stages of germ-line differentiation, the remaining spheres were cultured in PGCLC medium for 7 days. At day 10, isolated cells were assessed for VASA and DAZL again. In the conventional method, 23% of cells expressed positivity for VASA and 29% DAZL whereas direct spherification achieved a positivity rate of 43% for VASA ($P<0.005$) and 45% for DAZL ($P<0.005$). This increased expression in both VASA and DAZL signify the increased number of cells undergoing germline differentiation.

Additionally, BOULE was assessed for the presence of meiotic cells such as the spermatocyte. The conventional method yielded <1% BOULE positivity whereas in direct spherification, there was 10% positivity (*P<0.005*).

Direct spherifcation result shows that differentiation almost doubled in comparison to the conventional method, yielding more post-meiotic cells in the same amount of time

Limitations, reasons for caution: Despite a higher differentiation rate in direct spherification, these cells would still need to be tested for their fertilization potential. The ability to achieve fertilization, blastocysts and live pups would provide final proof and reliability of this method of neogametogenesis

Wider implications of the findings: Differentiating ESCs through direct spherification provides an alternative to studying intercellular relationships. This provides an opportunity to study spermatogenesis in more detail by replicating the microenvironment of the seminiferous tubule. Once embryo developmental competence of the *de novo* gamete is confirmed, this may open a new chapter in human reproduction

Trial registration number: N/A

O-102 Polymeric scaffold loaded with CD133+ BMDSCs for endometrial regeneration in Asherman's syndrome

N. Venkatesan[1], E. Fernandez Garcia[2], X. Santamaria Costa[2,3], C. Simon Valles[1,2,4]

[1]University of Valencia, Pediatrics- Obstetrics and Gynecology, Valencia, Spain ; [2]Igneomix Foundation, Research & Development, Paterna, Spain ; [3]Vall d´Hebron Institut de Recerca, Ginecologia, Barcelona, Spain ; [4]Harvard University, Obstetrics and Gynecology, Boston, U.S.A.

Study question: Can $CD133^+$ bone marrow-derived stem cells (BMDSCs) loaded in polyethylene glycol diacrylate (PEGda) and gelatin divide and decidualize?

Summary answer: Biocompatible porous PEGda and gelatin scaffold provides a three-dimensional environment for CD133+ cells to attach, divide, and decidualize *in vitro*.

What is known already: Intrauterine adhesions (IUA) develop due to acquired damages in the endometrium resulting in partial to complete endometrial dysfunction in the Asherman syndrome. Previous works from our group have demonstrated the engraftment of $CD133^+$ BMDSCs and its paracrine effect on endometrial proliferation, improved endometrial thickness and clinical outcome in murine and human models of Asherman syndrome (AS).

Study design, size, duration: Human CD133+ BMDSCs were obtained from refractory AS patients undergoing autologous cell therapy. Two different polymers PEGda and gelatin were analysed for their ability to form porous scaffold. CD133+ BMDSCs cell adhesion and division was analysed up to 14 days, and its differentiation upon 8-Br-cAMP was evaluated *in vitro* on day 5. *In vivo* biocompatibility was evaluated until week 5.

Participants/materials, setting, methods: Porous PEGda and gelatin scaffolds were synthesized by cryogelation. Porosity, interconnectivity, and its distribution were characterized by scanning electron microscopy (SEM) and micro-computed tomography. Cell adhesion, growth, and morphology were analysed by SEM and fluorescence microscopy while decidualization of the adhered cells were analysed by prolactin (PRL) and IGFBP1 secretion by ELISA and the mRNA expression levels by qPCR. Biocompatibility and degradation of the scaffolds were analysed by sub-cutaneous implantation in Sprague -Dawley rats.

Main results and the role of chance: The average pore size was higher in the case of gelatin (100 – 250 µm) compared to PEGda which had compact structure with through pores (25 – 150 µm) and thick walls. Cross-sectional analysis revealed, well interconnected pores in both polymers. There was no significant difference between the two polymers with respect to cell adhesion, and viability (> 80% in both the cases). There was a significant increase in the expression of mRNA levels of IGFBP1 with a fold change of 3 ± 2.25 (PEGda), and 10 ± 1.3 (gelatin) whereas for PRL it was 0.08 ± 0.82 (PEGda), and 0.39 ± 1.7 (gelatin) when treated with cAMP. Secretion of IGFBP1 (7.4 ± 4.5 pg/ml for PEGda and 8.5 ± 4 pg/ml for gelatin) and PRL (4.7 ± 1.8 pg/ml for PEGda and 5.6 ± 1.2 pg/ml for gelatin) also increased with the addition of cAMP. *In vivo*, PEGda degraded at a faster rate (~ 3 weeks) compared to gelatin (> 5 weeks) with no inflammatory reaction. Subcutaneous polymer degradation study was carried out to determine its degradation rate, its effect on inducing fibrosis, and to test its use as subcutaneous implant to aid in the regeneration of endometrium.

Limitations, reasons for caution: This is an *in vitro* study.

Wider implications of the findings: $CD133^+$ BMDSCs loaded inflatable PEGda and gelatin scaffold could be a potential alternative to deliver the cells locally for the repair of endometrial damage provoked by the iatrogenic destruction of the endometrial niche.

Trial registration number: Not applicable

human reproduction

ESHRE 2021 / Oral presentations

INVITED SESSION

SESSION 21: THE GENETIC DANCE IN HUMAN EMBRYOS

29 June 2021 Stream 1 08:30 - 09:30

O-020 Aneuploidy and mosaicism in human embryos: How correct detection may improve IVF clinical outcomes

S. Munne[1], **E. Fragouli**[1]

[1]*Overture Life, Research & Development, Alcobendas Madrid, Spain*

Abstract text

Study question: Can new next-generation sequencing (NGS) based strategies for preimplantation genetic testing of aneuploidy (PGT-A) improve clinical outcomes after assisted reproductive technology (ART)?

Summary answer: Recent randomised controlled trials (RCTs) suggest that NGS-based PGT-A strategies can improve clinical outcomes for older women. The clinical management of mosaic embryos remains controversial.

What is known already: There are two types of chromosome abnormalities present in embryos, meiotic arising mostly during oogenesis, and mitotic arising after fertilisation. Meiotic aneuploidies are present in all of the embryonic cells and in their vast majority are lethal. Conversely, mitotic abnormalities are present in only part of the embryonic cells with the remaining cells having a different cytogenetic constitution. This phenomenon is known as mosaicism. The sensitivity of NGS meant that mosaic aneuploidy became readily detectable in trophectoderm (TE) samples during PGT-A. The viability and clinical management of mosaic embryos has led to debates and controversies in the reproductive medicine field.

Study design, size, duration: The study involved an assessment of the impact of mosaic chromosome abnormalities to embryonic viability and clinical outcomes after ART cycles using PGT-A via NGS. A large number of embryos generated in IVF clinics in Europe and the USA was examined.

Participants/materials, setting, methods: Embryos were generated by couples referred for PGT-A due to various indications. All embryos were cultured to the blastocyst stage, and underwent a TE biopsy, followed by vitrification. TE samples were shipped to 6 reference PGT laboratories and analysed via the use of the same NGS platform. Mosaic chromosome abnormalities were scored according to validated thresholds set by the reference laboratories. The clinical management of mosaic embryos took place according to published guidelines.

Main results and the role of chance: Comparison of clinical outcomes seen after the transfer of mosaic embryos with those seen after the transfer of euploid embryos led to the following findings:

1. A significantly lower ongoing pregnancy rate (37% for mosaic embryos vs. 77% for euploid embryos, $p < 0.001$).
2. A significantly higher miscarriage rate (25% for mosaic embryos vs. 7% for euploid embryos, $p < 0.001$).
3. A significantly lower ongoing pregnancy rate per oocyte retrieval (37% for mosaic embryos vs. 63% for euploid embryos, $p < 0.001$).

Mosaic embryos with <40% abnormal cells in the TE sample had an OIR of 50% compared to 27% for mosaics with 40–80% abnormal cells in the TE, and 9% for complex mosaic embryos. Karyotyping of ongoing pregnancies resulting after the transfer of mosaic embryos demonstrated a normal chromosome constitution of the resulting foetuses.

Limitations, reasons for caution- Cytogenetic classification was based on TE samples removed from blastocysts during PGT-A analysis. As only a fraction of the cells from each embryo are tested, inevitably some mosaic embryos will be incorrectly classified fully euploid or aneuploid. However, this misclassification is expected to have little impact on the results.

Wider implications of the findings- The transfer of NGS-classified mosaic embryos was associated with poorer clinical outcomes compared to euploid embryos. However, the ongoing pregnancies resulting from mosaic transfers were euploid. NGS's ability to identify embryos of reduced viability has the potential to improve IVF clinical outcomes.

O-021 PGT-A and embryo selection: what can we actually agree upon?

D. Griffin[1]

[1]*University of Kent, Biosciences Building, Canterbury, United Kingdom*

INVITED SESSION

SESSION 22: FREEZE ALL FOR ALL? (DEBATE)

29 June 2021 Stream 2 08:30 - 09:30

O-022 Pro - FET: The future of IVF

C. Venetis[1]

[1]*University of New South Wales, UNSW Medicine, Sydney, Australia*

Abstract text

Assisted reproductive technology (ART) represents a relatively new but extremely dynamic field of medicine. Throughout its 40 year-old history, there has been a number of paradigm changes, all of which aimed to optimise the efficacy and safety of this technology. This presentation will cover all the scientific developments of the last few years that have now build the case for yet another paradigm change; the transition to predominantly frozen embryo transfers. Emerging knowledge on the effect of the supraphysiological levels of sex steroids, usually present during a stimulated cycle, on the probability of pregnancy combined with new strategies to completely eliminate the occurrence of ovarian hyperstimulation syndrome represent strong arguments in favour of frozen embryo transfer cycles. Dissociating ovarian stimulation from the embryo transfer, also allows for more intense stimulation, which can likely increase the cumulative live birth rate of a single aspiration and therefore reduce the need for future treatment. All these, combined with the encouraging evidence regarding the obstetric and neonatal outcomes of these pregnancies, render the option of the frozen-embryo transfer cycle quite compelling and a strong candidate as the future standard of care in IVF. This presentation will elaborate on these topics and provide guidance on the optimal strategy for frozen-thawed embryo transfer cycles so that the safety and efficacy of IVF are maximised.

O-023 Con - FET: An individualised approach is required

INVITED SESSION

SESSION 23: ADDED VALUE OF REPRODUCTIVE SURGERY IN ART

29 June 2021 Stream 3 08:30 - 09:30

O-024 Endoscopic management of the unexplained infertility, what does it add?

S. Gordts[1]

[1]*LIFE, Life Expert Centre, Leuven, Belgium*

Abstract text

Endoscopic management of the unexplained infertility, what does it add?

Stephan Gordts

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Unexplained infertility "strictu sensu" is not a diagnosis, but a description of a status where no causal factor is identified in a couple trying to conceive for at least one year. The more parameters are assessed, the more likely to identify an etiology, the less likely becomes "unexplained" infertility. Limiting the fertility exploration to indirect visualization techniques like ultrasound, HSG or HycoSy involves the risk of missing existing pathologies.

Uterus

Uterine volumetric abnormalities can be detected by indirect techniques, but information is lacking on the visualization of the endometrium in case of chronic endometritis and the presence of endometrial defects and hypervascularization areas as seen in patients with adenomyosis.

Tubo-ovarian

Even with the increased accuracy of indirect visualization techniques, lesions of minimal endometriosis and tubo-ovarian adhesions are not detected (*Table*). Tubal normality constitutes not only normal tubal patency but also normal tubal function. The importance of subtle tubal lesions is underestimated. Hydatid of Morgagni are detected in 38.1% in patients with infertility versus only in 16,7% in fertile women (*Gupta et al. JMIG 2017*).Removal of these lesions resulted in a spontaneous pregnancy rate of 58.7% versus 20.6 in the non-treated group (*Rasheed et al. EJOG Repr. 2011*).

Endometriosis

In a series of 107 patients with unexplained infertility and 3 failed IVF cycles (*Agni Pantou et al. J. Clin. Med. 2019)*laparoscopy revealed the presence of endometriosis in 57.97%, peri-adnexal adhesions in 23.3% and was normal in 18.69%. Also, in a group of patients with 3 failed IVF cycles and unexplained infertility *(Xiaoming Yu et al.Medicine 2019)* laparoscopy showed endometriosis in 57.7%, tubal abnormalities in 31.1% and adhesions in 33.3%. Laparoscopic correction of these pathologies did not only result in a spontaneous pregnancy rate of 35% but resulted also in a higher pregnancy rate after IVF compared to the non-treated control group.

Unexplained infertility hides frequently undiagnosed endometriosis. Endometrial BCL6 levels, a proto-oncogene where overexpression is associated with increased cellular proliferation and progesterone resistance, are increased in patients with endometriosis. In case of elevated BCL6 in patients with unexplained infertility, laparoscopy confirmed the presence of endometriosis in 93.8% (*Evans-Hoeker et al. 2016*). Abnormal BCL6 expression in a population with unexplained infertility reduced the chance of having a successful IVF treatment in 74% of the population *(Almquist et al. Fertil Steril 2017)*.

Transvaginal Hydro Laparoscopy

Direct endoscopic visualization remains important but due to the invasiveness, diagnostic standard laparoscopy is frequently postponed or omitted in the exploration of the infertile patient. The technique of transvaginal hydro-laparoscopy allows in a minimal invasive way the inspection of the pelvis. In a consecutive series of 2288 patients without obvious pelvic pathology, findings were normal in 49.3%, endometriosis was diagnosed in 15.9% and tubal pathology in 14.5% of the patients *(Gordts et al. FVV 2021)*. The rate of failed access was 1% and the complication rate 0.74%. Causing a minimal ovarian trauma, treatment of these early endometriotic lesions resulted in a spontaneous pregnancy rate of 73.2%.

Conclusion: The inappropriate use of "unexplained infertility" by omitting the diagnostic endoscopy in the exploration of the infertile patient, can hide undiagnosed and treatable pathology, jeopardizing possibilities for patients for a spontaneous conception and can be responsible for reduced pregnancy rates after IVF.

O-025 Value of hysteroscopy

S. Haimovich[1]

[1]*Director of the Gynecology Ambulatory Surgery and Hysteroscopy Unit. Clinical Assistant Professor at the Rappaport Med School- Technion, Obstetrics and Gynecology at the Hillel Yaffe Medical Center, Hadera, Israel*

Abstract text

The 3 main characters in any ART are the uterus, the endometrium, and the embryo. With the rutinary use of 2D US and especially 3D US in infertile patients we are able to assess most of the uterine anomalies and intrauterine pathologies.

Until recently, the assessment of the endometrial cavity with hysteroscopy was reserved only for cases of IVF failure, as a complementary evaluation to ensure that nothing was missed during the ultrasound scan.

We also need to remember how hysteroscopy was performed in the past and, unfortunately, in some cases even today. In the old days, to assess the endometrial cavity, it was required to take the patient to the operating room and under general anesthesia, dilate the cervix to then introduce a large diameter hysteroscope only for diagnostic purposes.

The prevalent working model in ART today is in close collaboration between Fertility specialists, Ultrasound and Hysteroscopy units in order to improve patient's outcome.

In our center, a diagnostic hysteroscopy is performed as part of the diagnostic workup of the infertility patient. It is performed in office setting and without anesthesia, by the staff of the reproduction and infertility unit.

When we look at hysteroscopy as an in-office procedure and no longer as a procedure performed in operating room, we appreciate that it is something that all infertility patients can benefit from.

The right question to ask now would be "Is there a benefit in performing a hysteroscopy to all our patients?".

Ultrasound is not perfect, especially when evaluating the endometrium. The gold standard and the only modality that we have to assess the endometrium with direct visualization is hysteroscopy. Chronic endometritis, adhesions and adenomyosis are only a few examples of what can be assessed by direct vision of the endometrial cavity.

The advantages of a simple, inexpensive, office procedure such as hysteroscopy outweigh any other consideration against it.

During my talk all these points will be presented helping to understand why hysteroscopy is becoming an indispensable tool in every assisted fertility unit.

INVITED SESSION

SESSION 24: EXCHANGE SESSION - FERTILITY SOCIETY OF AUSTRALIA

29 June 2021 Stream 4 08:30 - 09:00

O-026 Advanced Glycation Endproducts: A new player in obesity related infertility

J. Hutchison[1,2], **T.T. Truong**[3], **T.A. Egell**[2], **L.A. Salamonsen**[1,2], **D.K. Gardner**[3], **J. Evans**[2]

[1]*Monash University, Molecular and Translational Science, Melbourne, Australia ;*
[2]*Hudson Institute of Medical Research, Centre for Reproductive Health, Melbourne, Australia ;*
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Abstract text

Globally, 39% of the adult population is overweight or obese, with the prevalence of obesity following an upward trajectory over the recent decades (WHO). Up to 30% of women of reproductive age in Western countries are obese before conception, and obese women experience higher rates of infertility and pregnancy complications than lean women; however, the mechanisms underpinning obesity-related infertility are poorly understood. Advanced Glycation Endproducts (AGEs) are a proinflammatory modification of proteins exposed to sugars, formed through the Maillard reaction. AGEs are elevated four-fold in the uterine fluid of obese, infertile women, compared to lean. AGEs equimolar to those in the obese microenvironment negatively impact the functions of endometrial epithelial and stromal cells, and adhesion and invasion of trophoblast cells, reducing the potential for successful maternal-fetal interactions (Antoniotti et al., 2018). This research further investigated preimplantation embryo development and endometrial cell functions in the presence of AGEs equimolar to those in obese uterine fluid.

Altered local environments in very early life can set offspring up for a lifetime of health or disease (DoHAD); thus, uterine AGEs may contribute to the prevalence of non-communicable disease in children of obese parents. Preimplantation mouse embryos were cultured *in vitro* with AGEs equimolar with uterine fluid concentrations from lean and obese women, and their development and implantation potential assessed. "Obese" AGEs did not impact the proportion of embryos reaching blastocyst stage by day 4, but significantly reduced the proportion of blastocysts hatching by day 5 ($P < 0.01$). AGEs equimolar with the obese uterine environment detrimentally impacted trophectoderm formation and function: reduced trophectoderm cell number ($P < 0.01$), reduced outgrowth on fibronectin (indicative of reduced implantation potential, ($P < 0.01$), but did not increase cell apoptosis (TUNEL assay). RAGE antagonism, but neither metformin nor antioxidants, improved trophectoderm cell number. Thus, obesity-associated AGEs link obesity and reduced fertility through poor placentation potential of embryos (Hutchison et al, 2020).

Endometrial epithelial cell function was examined in the presence of lean and obese concentrations of AGEs. Obese AGEs significantly reduced the rate of proliferation (xCelligence real time cell analysis) of the endometrial epithelial cell line ECC-1 versus lean AGEs ($P = 0.04$). Antioxidants successfully restored the rate of proliferation in the presence of obese AGEs ($P = 0.7$ versus lean AGEs). Subsequently, human endometrial epithelial organoid culture was utilised as a more physiologically relevant experimental paradigm. When cultured as organoids, primary endometrial epithelial cells were functionally responsive to obesity-associated AGEs, expressing both RAGE and TLR4. The morphology of organoids in culture was not impacted by the presence of obese AGEs versus lean; however, the proliferation of epithelial cells retrieved from organoid culture was altered by obese AGEs versus lean. Obese AGEs also increased the secretion of proinflammatory CXCL16 versus vehicle control ($P = 0.04$) while increased secretion of other proinflammatory cytokines and chemokines including TNFa approached significance in the presence of obese AGEs. As the inflammatory milieu is altered in the uterine fluid of infertile women, elevated AGEs may promote an infertile endometrial inflammatory environment.

AGEs link obesity and reduced fertility, being detrimental to preimplantation embryo development and endometrial cell function when present at concentrations equal to those in obese uterine fluid. Antioxidants and RAGE antagonism provide beneficial effects to cell function in the presence of obesity-associated AGEs. This research provides evidence supporting AGEs as a factor contributing to obesity related infertility, and as an emerging frontier for reproductive health. Clinically, reduction of uterine AGEs may improve fertility for obese couples wishing to conceive.

Antoniotti et al (2018). Hum Rep. 33(4), 654-665. PMID: 29471449

Hutchison et al (2020). RBMO. 41(5), 757-766. PMID: 32972872

SELECTED ORAL COMMUNICATIONS

SESSION 25: SUSTAINABLE ART: ADAPTATION TO A CHANGING WORLD

29 June 2021 Stream 1 10:00 - 11:30

O-103 The impact of the COVID-19 pandemic on infertility and endometriosis patients in the Netherlands: The use of telemedicine, quality of life and patient-centeredness

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Study question: How do infertility patients, endometriosis patients and health care providers rate the virtual care that was provided during the first lockdown of the COVID-19 pandemic?

Summary answer: Patients and health care providers rate telephone- and video consultations as good alternatives during the pandemic but it cannot replace future physical consultations.

What is known already: Virtual alternatives to regular care such as telephone and video consultations are gaining more attention as replacement for physical consultations and are ideal for use in a social distancing situation as the COVID-19 pandemic. However, infertility and endometriosis patients often rely on physical consultations for reassurance as well as for treatments such as artificial reproductive technology and surgery. Not being able to receive these reassurances and treatments may cause stress especially as infertility patients are known to experience a high sense of urgency to obtain treatment. For patients with endometriosis, regular follow-up visits are important for continuity of care.

Study design, size, duration: A cross-sectional cohort study was performed, including 555 patients and 101 health care providers in the field of infertility and endometriosis in the Netherlands. Online questionnaires were sent between May and October 2020.

Participants/materials, setting, methods: Patients with infertility and endometriosis patients from a university hospital and members of the respective national patients organizations, as well as health care providers in the fields of infertility and endometriosis were asked to participate. The questionnaires consisted of demographics, appraisal of telephonic and video consultations (TCs and VCs) and assessment of fertility related quality of life (FertiQoL) and patient-centeredness of endometriosis care (ENDOCARE).

Main results and the role of chance: The questionnaires were completed by 374 infertility patients, 181 endometriosis patients and 101 health care providers. 75.9% of the infertility patients, 64.8% of the endometriosis patients and 82.7% of the health care providers rated TCs as a good alternative for physical consultations during the COVID-19-pandemic. Only 21.3%, 14.8% and 21.3% rated TCs as a good replacement of physical consultations in general. 76.6% and 35.9% of the infertility and endometriosis patients reported to experience an increase in stress due to the altered care during the COVID-19 pandemic. 38.7% and 58.0% reported to have received sufficient information from their care givers. Infertility patients scored lower on the FertiQoL than the reference population, while the ENDOCARE results of endometriosis patients were comparable to the reference.

Limitations, reasons for caution: This study was limited to the Dutch population. As the organization of infertility care varies internationally, the results will not be directly applicable to other countries or health care systems.

Wider implications of the findings: Virtual care options are a good alternative for infertility and endometriosis patients in situations where physical consultations are not possible. Self-reported stress is especially high in infertility patients during the COVID-19-pandemic. Health care providers should provide more information to patients in order to increase their ability to cope with stress.

Trial registration number: N/A

O-104 Low SARS-CoV-2 positivity rate in women included in ART programs following the recommendations of the Spanish scientific societies on reproduction (SEF/ASEBIR)

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Study question: What is the SARS-CoV-2 positivity rate following the Spanish Fertility Society (SEF) / Association for the Study of Reproductive Biology (ASEBIR) screening recommendations?
Summary answer: The SARS-CoV-2 positivity rate in the centers following the SEF/ASEBIR screening recommendations was 0.316% after the first survey and 0.364% after the second one
What is known already: Due to the Sars-Cov-2 pandemic, all the Medical Assisted Reproduction (MAR) centers in Spain had to interrupt their activity most of the time during the first pandemic wave. On April 27th activity was restarted, and SEF and ASEBIR jointly elaborated a guide describing their SARS-CoV-2 screening recommendations for MAR centers. This document aims to achieve a safe environment for patients and staff. It includes the possibility of screening patients through a targeted clinical interview and the use of reverse-transcriptase polymerase chain reaction (RT-PCR). The aim of this study is to quantify the SARS-CoV-2 positivity rate based on these recommendations.
Study design, size, duration: National multicenter cross-sectional study. Information was gathered from centers using an anonymous survey asking for aggregated data about the number of positive cases among screened patients, sent twice. The first survey covered the period April 27th - June 30th. Second survey covered July 1st - August 31st. Response rates among centres were 9% (29/319) and 6% (20/319), respectively. This study includes 2,695 and 4,068 screenings performed in the first and the second survey, respectively.
Participants/materials, setting, methods: The SEF/ASEBIR recommendations describe two screening strategies. Strategy (a) consists in a targeted clinical interview (TCI) evaluating clinical symptoms and exposure risk, first before starting the cycle, and before egg-retrieval, intrauterine insemination (IUI), and/or embryo transfer (ET). Suspicious cases could be confirmed by further RT-PCR testing. Strategy (b) consists in conducting the same first TCI, and a systematic RT-PCR testing before the medical procedure in all patients. All patients in both strategies have a TCI.
Main results and the role of chance: In the 1st survey, 1,177 screenings and 919 RT-PCR (78.07%) were performed before the egg-retrieval. One patient with a negative TCI and positive RT-PCR was detected, and the cycle was cancelled. 1,518 screenings and 1,161 RT-PCRs (76.48%) were performed before the ET / IUI. Two patients with a positive TCI were detected, one did not perform a RT-PCR, while the other resulted in a positive RT-PCR. Both cycles were cancelled. Besides, 5 patients with negative TCI performed a RT-PCR with a positive result; all 5 were cancelled. Overall, the SARS-CoV-2 positivity rate was 8/2533 (0.316%), of which 7/2533 (0.276%) were identified by RT-PCR testing.

The 2nd survey included 1,376 screenings and 1,009 RT-PCR (73.32%) performed before the egg-retrieval. Four patients with negative TCI and further positive RT-PCR were detected, and their cycle was cancelled. 2,692 screenings and 2,134 RT-PCR (79.27%) were performed before ET / IUI. Two patients had a positive TCI, one with a negative, the other with a positive RT-PCR testing; both cycles were cancelled. Besides, 8 patients with negative TCI, but positive RT-PCR testing, were detected and their cycles cancelled. Overall, the SARS-CoV-2 positivity rate was 14/3846 (0.364%), of which 13/3846 (0.338%) after positive RT-PCR testing.
Limitations, reasons for caution: The criteria for performing the RT-PCR testing were not the same in all MAR Centres or even in the same centre at different times. Due to the low response rate of the study, we should not extend these results to all the MAR Centres in Spain.
Wider implications of the findings: The results of the surveys suggest that the SEF / ASEBIR recommendations could be a good screening strategy for SARS-Cov-2 at MAR Centres. Further survey collected at different times of the pandemic are warranted, including new strategies for screening as antigen tests or vaccination status.
Trial registration number: Not applicable

O-105 High prevalence of obesity and polycystic ovary syndrome in an online community seeking assistance with fertility

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Study question: What are the characteristics of a large online community of women trying to conceive and what are the factors that predict infertility in this group?
Summary answer: The cohort was not characterized by increased age, though age, obesity and PCOS strongly correlated with fertility. PCOS may be under-recognized within some ethnic groups.
What is known already: Obesity, polycystic ovary syndrome (PCOS), and age are among the primary predictors of infertility in women. However, most studies assessing reproductive health employ populations who have sought medical assistance for infertility or associated disorders, potentially leading to populations more likely to exhibit disorders such as PCOS or to be of increased age. Characterization of factors affecting fertility in a general population may highlight epidemiological influences that should be better addressed to aid women trying to conceive.
Study design, size, duration: This study employed users of the mobile application, FertilityAnswers, for people searching for answers to fertility problems. Users answered a short survey describing themselves, their fertility history and goals. Recruitment for this study began in March 2017 and ended in January 2021. 61814 participants downloaded the application during this period, and 56878 at least provided their age. The primary inclusion criteria were that study participants be US females over eighteen years of age.
Participants/materials, setting, methods: Regression models estimated beta coefficients and corresponding confidence intervals. Multivariable models determined independence of variables. To model PCOS, the available data in January 2020 was split randomly into discovery and validation subsets. Missing value imputation using random forests was performed in the discovery dataset. Minimal feature selection used a linear regression model penalized with a lasso and elastic net. The model was then validated on samples collected after the model was trained and tested.
Main results and the role of chance: Age was a significant predictor of fertility in this study (p<1x10-10). However, the distribution of age in the cohort was very similar to that of women at first birth in the United States, therefore we did not observe the majority of study participants to be of an age where in a typical clinical setting age-related concerns would be addressed (i.e., approaching 35 years). Using National Center for Health Statistics data, the mean age of 1,433,604 women at first birth in 2018 in the US was younger than the study population (restricted to those without children) by only one month. Obesity was of increased prevalence in this cohort, with 55% being obese, compared to 37% in an age-matched US population. Participants reported a variety of fertility-related disorders, with polycystic ovary syndrome (PCOS) being most

prevalent (19.0%), followed by endometriosis (6.0%). Prediction of PCOS, performed by modeling on training and test sets (10476 and 5312 participants) and then validating with an additional 21097 participants collected after the model creation, found that African-Americans and Latino members of this cohort had a lower self-reported rate of the disorder than was anticipated by the model, in contrast to those of Asian or European descent.

Limitations, reasons for caution: All health data was self-reported. Additionally, as this is the initial survey of this population, no a priori hypotheses were made as to the expected relationships to be observed. Instead, all associations were examined, and measurements of false discovery rate were estimated.

Wider implications of the findings: We found that women were seeking answers about infertility at ages coincident with that of their peers achieving first pregnancies. ART is often not a first-line treatment for women of this age, but there may be a disconnect between traditional clinical response to this group and their desire for assistance.

Trial registration number: not applicable

O-106 Non-donor IVF treatment cycles in the UK need to be re-evaluated against the 1.625 million oocytes retrieved

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Study question: Are IVF clinics collecting too many oocytes per retrieval procedure?

Summary answer: IVF cycles performed in the UK appear to be retrieving far too many oocytes, most of which may never be used and are probably discarded.

What is known already: For justifying IVF with low AMH, older women, poor responders, the Bologna and POSEIDON consensuses were developed. The positive linear correlation between cumulative live birth rates and numbers of oocytes collected is well established, thereby focussing intensely on stimulation regimes and the growth of FER cycles and oocyte freezing activities. The associated risk of OHSS is well-known. However, over-stimulation practices and numbers of oocytes retrieved within IVF remain unknown as is the impact on patients' health, emotional and financial welfare. This UK dataset uniquely reveals numbers of oocytes retrieved against IVF cycles undertaken, and which may well reflect global practices.

Study design, size, duration: This is a retrospective observational cohort study of oocyte retrieval procedures for non-donor IVF cycles in the UK between 2015 and 2018. Data were obtained from UK HFEA under the Freedom of Information Act 2000. For fresh oocytes, data were obtained for the number of cycles retrieving 1-5, 6-15, 16-25, 26-49, 50-59, and 60+ oocytes. The number of cycles that led to no oocytes was obtained as well as data on the utilisation of oocytes.

Participants/materials, setting, methods: The data from the HFEA covers up to 86 UK IVF clinics undertaking non-donor IVF. IVF clinics are legally obliged to provide IVF dataset as part of the licence requirement. The unbiased data was gathered independently by HFEA staff under the Freedom of Information Act 2000. Specifically, the number of treatment cycles with; 0, 1-5, 6-15,16-25, 26-49, 50-59, and 60+ oocytes retrieved for each year was requested. Additional limited data could be gained.

Main results and the role of chance: For 2015-2018 there were 172341 fresh oocyte retrieval cycles, where 10148 (5.9%) cycles from 9439 patients did not yield any oocytes. In this period, 42574 cycles (24.7%), 91797 cycles (53.3%), 23794 cycle (13.8%) and 3970 cycles (2.3%) yielded 1-5 oocytes, 6-15 oocytes, 16-25 oocytes, 26-49 oocytes respectively, while 58 cycle (0.033%) yielded over 50 oocytes. The data was accountable by 5-85 clinics and the outcomes and patterns remained uniform across the 4 years. The main desired oocyte yield of 6-15 oocytes occurred in 53.3% of IVF cycles distributed evenly across the clinics. However, 16.1% of cycles were associated with 16-49 oocytes retrieved per IVF cycle, while 58 (0.03%) cycles led to greater than 50 oocytes retrieved. The maximum number of oocytes collected was not provided by the HFEA due to technical reasons.
The total number of oocytes collected over 4-years numbered 1,624,912 oocytes from 147274 women yielding on average 11 oocytes per patient. These oocytes were fertilised to yield 931,265 embryos (57.3% converted). The fate of 42.7% oocytes remains unknown. Of the embryos created, 209,080 (22.4%) were transferred over 172,333 cycles, while 219,563 (23.6%) embryos frozen and the fate of 53.97% of embryos remained unaccounted for.

Limitations, reasons for caution: This retrospective analysis spans 4 years in which stimulation regimes, patient characteristics, or outcomes were unavailable. Only a qualitative analysis is possible with the HFEA dataset, but the corresponding data is unique and of public interest. The outcome of unaccounted oocytes appears a limitation in the regulatory body data set.

Wider implications of the findings: This unique observation on IVF clinics practices suggests that the high oocyte number per retrieval procedure needs re-evaluation. In particular, this needs to focus on the side-effects, including OHSS and procedure-related complications. In addition, the outcome and cost of unused frozen oocytes need to be established.

Trial registration number: not applicable

O-107 Searching for the optimal number of oocytes to reach a life birth following in vitro fertilization: a systematic review with meta-analysis

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Study question: To investigate the relationship between the number of oocytes and both the live birth rate after fresh embryo transfer and the cumulative live birth rate.

Summary answer: Above a 15-oocyte threshold, live birth rate (LBR) following fresh transfer plateaus, whereas a continuous increase in cumulative live birth rate (CLBR) is observed.

What is known already: Several lines of evidence indicate that number of oocytes represents a key point for in vitro fertilization (IVF) success. However, consensus is lacking regarding the optimal number of oocytes for expecting a live birth. This is a key question because it might impact the way practitioners initiate and adjust COS regimens.

Study design, size, duration: A systematic review and meta-analysis was performed. MEDLINE, EMBASE, and Cochrane Library were searched for studies published between January 01, 2004, and August 31, 2019 using the search terms: "(intracytoplasmic sperm injection or icsi or ivf or in vitro fertilization or fertility preservation)" and "(oocyte and number)" and "(live birth)".

Participants/materials, setting, methods: Two independent reviewers carried out study selection, quality assessment using the adapted Newcastle-Ottawa Quality Assessment Scales, bias assessment using ROBIN-I tools, and data extraction according to Cochrane methods. Independent analyses were performed according to the outcome (LBR and CLBR). The mean-weighted threshold of optimal oocyte number was estimated from documented thresholds, followed by a one-stage meta-analysis on articles with documented or estimable relative risks.

Main results and the role of chance: After reviewing 843 records, 64 full-text articles were assessed for eligibility. A total of 36 studies were available for quantitative syntheses. Twenty-one and 18 studies were included in the meta-analyses evaluating the relationship between the number of retrieved oocytes and LBR or CLBR, respectively. Given the limited number of

investigations considering mature oocytes, association between the number of metaphase II oocytes and IVF outcomes could not be investigated. Concerning LBR, 7 (35.0%) studies reported a plateau effect, corresponding to a weighted mean of 14.4 oocytes. The pooled dose-response association between the number of oocytes and LBR showed a non-linear relationship, with a plateau beyond 15 oocytes. For CLBR, 4 (19.0%) studies showed a plateau effect, corresponding to a weighted mean of 19.3 oocytes. The meta-analysis of the relationship between the number of oocytes and CLBR found a non-linear relationship, with a continuous increase in CLBR, including for high oocyte yields.
Limitations, reasons for caution: Statistical models show a high degree of deviance, especially for high numbers of oocytes. Further investigations are needed to assess the generalization of those results to frozen mature oocytes, especially in a fertility preservation context, and to evaluate the impact of female age.
Wider implications of the findings: Above a 15-oocyte threshold, LBR following fresh transfer plateaus, suggesting that the freeze-all strategy should probably be performed. In contrast, the continuous increase in CLBR suggests that high numbers of oocytes could be offered to improve the chances of cumulative live births, after evaluating the benefit–risk balance.
Trial registration number: Not applicable

O-108 Opinion and perceptions about how new family models conceived through Assisted Reproduction Techniques and parenthood differ between different generations in Argentina.

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Study question: What is the degree of acceptance of new family models using Assisted Reproductive Techniques and what is the childbearing perception among different generations in Argentina
Summary answer: The youngest accept different family conformations through fertility treatments more than olders. They also consider, in greater proportion, that happiness is not linked to childbearing
What is known already: (1). Human reproduction changed dramatically in the last 40 years, with the development of Assisted Reproductive Treatments (ART) (2). In accordance with the new family models, gender diversity, self-acceptance and social openness, unacceptable until recently, are today a new reality. Since 2013, in Argentina, fertility treatments are regulated under National Law No. 26.862, allowing full access to ART regardless of marital status or sexual orientation. (3). However, there is still a legal vacuum around uterine surrogacy, which hinders treatment particularly in male homosexual couples.
Study design, size, duration:
This is a cross-disciplinary descriptive study based on data obtained from an online self-administered survey. We surveyed a sample of 1800 people from the general population during June 2020.
Participants/materials, setting, methods: A structured and self-administered survey was carried out through OPINAIA (research consultant). Participants answered voluntarily an anonymous online questionnaire. We stratified our population in 4 groups according to different generations that represent the Argentinean population: Centennials (18-25 years), Millennials (26-35 years), Gen X (36-49 years), Baby Boomers (more than 50 years), respectively. We also stratified our sample by gender, socioeconomic level, and geographic location.
Main results and the role of chance: The data obtained in our survey showed that 92% of our population expressed an agreement to the use of ART for heterosexual couples, 76% for single women, 65% for single men, 62% for female couples and 59% as to male couples. However, when stratifying by generations, we observed that the youngest showed a clear tendency to accept the new family conformation models with respect to the oldest ones.

When comparing by generations, Centennials showed a strong agreement for single women (63%), single men (52%), female couples (60%) and male couples (58%). However, Baby Boomers express agreement on single women (33%), single men (24%), female partners (24%), male partners (22%). So, our data reveals that the prejudices concerning the different family conformations models are less among the youngest.

We also observe a tendency towards believing that happiness is not related to parenthood by the younger (Centennials 75%, Millennials 67%, X Generation 64%, Baby Boomers 60%).

This is a representative sample of the Argentinean populations, based upon official National census. Thus, it accurately represents the local distribution concerning age, gender, socioeconomic level, and geographic location.
Limitations, reasons for caution: Our investigation is a descriptive and observational study
Wider implications of the findings: This is the first study about new family models in Latin America. The study sample represents the national population and reflects clearly social trends. Therefore, it allows predicting future scenarios for policy makers to plan effective education strategies and to consider the distribution of public health funds for fertility treatments.
Trial registration number: not applicable

INVITED SESSION
SESSION 26: LIVE SURGERY SESSION

29 June 2021 Stream 2 10:00 - 13:00

O-027 Placental remnant and the use of shaver

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O-028 Uterine congenital anomalies

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O-029

R.L. Campo[1]

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O-030 Subtle tubal lesions

A, Watrelot[1]

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O-071 The ovarian endometriotic cyst

M. Nisolle[1]

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Abstract text
"The ovarian endometriotic cyst"

Ovarian endometriomas affect 17 to 44% of women with endometriosis, and are often associated with pelvic pain and infertility.

Patients suffering from endometriosis frequently present an already reduced ovarian reserve, assessed by AMH dosage or by antral follicular count during TVS.

Pain and infertility are the main indications for endometrioma surgery which is a complex procedure as endometriosis leads to inflammation around the lesions, causing fibrosis.

Three main surgical procedures have been described: the ovarian cystectomy, the endometrioma ablation or the combined technique.

During the cystectomy, after ovarian mobilization and adhesions lifting, an incision of the cortex is realized to find a cleavage plane between the cyst wall and the ovarian cortex. Traction and countertraction movements are performed to carefuly dissociate the cyst from the ovarian cortex. It is crucial to handle the ovarian tissue as atraumatically as possible. With this technique, the cyst wall as well as the surrounding fibrosis are excised with the risk of oocytes removal responsible for decreased ovarian reserve.

The ablative surgery is defined by the fenestration and vaporization of the endometrioma cyst. The ablation is carried out using a laser or plasma energy

or electrocoagulation. Once the endometrial cyst has been emptied of its contents, the entire internal surface of the endometrioma must be sprayed or evaporated using the different chosen techniques. Where feasible, the cyst may be turned inside out to facilitate further treatment.

The combined technique associates partial cystectomy (80-90%) and ablation of the 10-20% remaining endometrioma. This method is especially useful while operating large endometriomas. It prevents excessive bleeding or damage to the ovarian tissue.

In cases of large ovarian endometrioma, the three-step approach has been proposed, consisting on an opening and drainage of the cyst followed by a 3 months' administration of Gn-RH agonists in order to reduce its diameter and vascularization. A second surgical procedure is then scheduled to ablate the remaining cyst wall.

In conclusion, it is crucial to keep in mind that endometriosis and especially the presence of endometrioma reduce fertility whereas in the majority of cases, the ovarian reserve is already diminished in relation to the patient's age. Ovarian preservation must be one of our priorities in young patients of childbearing age and it is therefore really important to carry out surgeries that are as atraumatic as possible.

Trial registration number:
Study funding:
Funding source:

SELECTED ORAL COMMUNICATIONS

SESSION 27: THE ART OF OVARIAN STIMULATION - NEW STUDIES ON THE BLOCK

29 June 2021 Stream 3 10:00 - 11:30

O-109 A first dose-response trial investigating the effects of adding choriogonadotropin beta to follitropin delta in women undergoing ovarian stimulation in a long GnRH agonist protocol

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Study question: Does addition of choriogonadotropin beta (CG beta) to follitropin delta increase the number of good-quality blastocysts following ovarian stimulation in a long GnRH agonist protocol?

Summary answer: At the doses investigated, the addition of CG beta reduced the number of intermediate follicles and decreased the number of oocytes and blastocysts.

What is known already: CG beta is a new recombinant hCG (rhCG) molecule expressed by a human cell line (PER.C6[a]) with a different glycosylation profile compared to urinary hCG or rhCG derived from a Chinese Hamster Ovary (CHO) cell-line. In the first-in-human trial, the CG beta pharmacokinetics were similar between men and women. In women, the area under the curve (AUC) and the peak serum concentration (C_{max}) increased dose proportionally following single and multiple daily doses. In men, a single dose of CG beta provided higher exposure with a longer half-life and proportionately higher testosterone production than rhCG derived from a CHO cell line.

Study design, size, duration: Placebo-controlled, double-blind, randomised trial (RAINBOW) to explore the efficacy and safety of CG beta as add-on treatment to follitropin delta in women undergoing COS in a long GnRH agonist protocol. The primary endpoint was the number of good-quality blastocysts (grade 3 BB or higher, Gardner and Schoolcraft, 1999). Subjects were randomised to receive either placebo or 1, 2, 4, 8, or 12 µg CG beta added to the daily individualised follitropin delta dose during COS.

Participants/materials, setting, methods: In total 619 women (30-42 years) with AMH levels between 5 and 35 pmol/L were randomized in equal proportions to the six treatment groups. All subjects were treated with an individualised dose of follitropin delta determined based on AMH (Elecsys AMH Plus Immunoassay) and body weight. Triggering was performed when 3 follicles were ≥17 mm but no more than 25 follicles ≥12 mm were reached

Main results and the role of chance: The incidence of cycle cancellation (range 0%-2.9%), total follitropin delta dose (mean 112 µg) and duration of stimulation (mean 10 days) were similar across the groups. A reduced number of intermediate follicles (12 to 17 mm) and fewer oocytes (mean range 9.7 to 11.2) were observed for all doses of CG beta compared to the follitropin delta only group (mean 12.5). The mean number of goodquality blastocysts was 3.3 in the follitropin delta group and ranged between 2.1 and 3.0 across the CG beta groups. The incidence of transfer cancellation was higher in the 4, 8 and 12 µg group, mostly as no blastocyst was available for transfer. In the group receiving only follitropin delta, the ongoing pregnancy rate (10-11 weeks after transfer) was high i.e. 43% per started cycle vs 28-39% in CG beta groups and 49% per transfer vs 38-50% in the CG beta groups. In line with the number of collected oocytes, the OHSS incidence was overall lower following follitropin delta with CG beta than following follitropin delta only treatment. Regardless of the dose, CG beta was safe and well-tolerated with low risk of immunogenicity.

Limitations, reasons for caution: The effect of the unique glycosylation of CG beta and the associated potency implications in women were not known prior to this trial. Further studies will be needed to evaluate potentially lower doses of CG beta for this and/or different indications.

Wider implications of the findings: The high ongoing pregnancy rate in the follitropin delta group supports the use of individualised follitropin delta dosing in a long GnRH agonist protocol. The differential potency of CG beta may have impaired the growth of intermediate follicles with the investigated doses without affecting the ongoing pregnancy rates per transfer.

Trial registration number: NCT03564509

O-110 A randomised, controlled, assessor-blind trial assessing clinical outcomes of individualised dosing with follitropin delta in Asian IVF/ICSI patients

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Study question: To evaluate the efficacy and safety of individualised dosing with follitropin delta versus conventional dosing with follitropin alfa in an Asian population undergoing ovarian stimulation.

Summary answer: Individualised dosing with follitropin delta results in significantly higher live birth rate and fewer early OHSS and/or preventive interventions compared to conventional follitropin alfa dosing.

What is known already: Previous randomised controlled trials conducted in Europe, North- and South America mainly including Caucasian IVF/ICSI patients as well as in Japan have demonstrated that ovarian stimulation with the individualised follitropin delta dosing regimen based on serum AMH level and body weight modulated the ovarian response and reduced the risk of OHSS without compromising pregnancy and live birth rates.

Study design, size, duration: Randomised, controlled, assessor-blind trial conducted in 1,009 Asian patients from mainland China, South Korea, Vietnam and Taiwan, undergoing their first IVF/ICSI cycle. Randomisation was stratified by age (<35, 35-37, 38-40 years). The primary endpoint was ongoing pregnancy assessed 10-11 weeks after transfer (non-inferiority limit -10.0%; analysis adjusted for age strata). Patients <35 years underwent single embryo transfer if a good-quality embryo was available, otherwise double embryo transfer. Patients ≥35 years underwent double embryo transfer.

Participants/materials, setting, methods: Follitropin delta (Rekovelle, Ferring Pharmaceuticals) daily treatment consisted of a fixed dose individualised according to each patient's initial AMH level (<15 pmol/L: 12 µg; ≥15 pmol/L: 0.19 to 0.10 µg/kg; min-max 6-12 µg) and body weight. Follitropin alfa (Gonal-f, Merck Serono) dose was 150 IU/day for the first five days with subsequent potential dose adjustments according to individual response. A GnRH antagonist protocol was applied. OHSS was classified based on Golan's system.

Main results and the role of chance: The ongoing pregnancy rate was 31.3% with follitropin delta and 25.7% with follitropin alfa (adjusted difference 5.4% [95% CI: -0.2%; 11.0%]). The live birth rate was significantly higher at 31.3% with follitropin delta compared to 24.7% with follitropin alfa (adjusted difference 6.4% [95% CI: 0.9%; 11.9%]; p<0.05). Live birth rates per age stratum were as follows for follitropin delta and follitropin alfa; <35 years: 31.0% versus 25.0%, 3537 years: 35.3% versus 26.7%, 38-40 years: 20.0% versus 14.3%. Early OHSS risk, evaluated as the incidence of early OHSS and/or preventive interventions, was significantly (p<0.01) reduced from 9.6% with follitropin alfa to 5.0% with follitropin delta. The number of oocytes was 10.0±6.1 with follitropin delta and 12.4±7.3 with follitropin alfa. Individualised follitropin delta dosing compared to conventional follitropin alfa dosing resulted in 2 more oocytes (9.6±5.3 versus 7.6±3.5) in potential low responders (AMH <15 pmol/L) and 3 fewer oocytes (10.1±6.3 versus 13.8±7.5) in potential high responders (AMH ≥15 pmol/L). Among patients with AMH ≥15 pmol/L, excessive response occurred less frequently with individualised than conventional dosing (≥15 oocytes: 20.2% versus 39.1%; ≥20 oocytes: 6.7% versus 18.5%). Total gonadotropin dose was reduced from 109.9±32.9 µg with follitropin alfa to 77.5±24.4 µg with follitropin delta.

Limitations, reasons for caution: The trial only covered the clinical outcome of one treatment cycle with fresh cleavage-stage embryo transfers.

Wider implications of the findings: The present trial implies that in addition to reducing the early OHSS risk, individualised dosing has the potential to improve the take-home baby rate in fresh cycles across all ages and with a lower gonadotropin consumption. The benefits in outcomes appear to be explained by the modulation of ovarian response.

Trial registration number: NCT03296527

O-111 The DuoStim strategy shortens the time to obtain an euploid embryo in poor prognosis patients: a non-inferiority, randomized controlled trial

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Study question: Is there any difference in the time to obtain euploid embryos from poor prognosis patients who performed two conventional cycles versus double stimulation (DuoStim) in the same cycle?

Summary answer: DuoStim showed similar ovarian response and *in vitro* fertilization (IVF) laboratory outcomes while shortening the time to obtain an euploid embryo in poor prognosis patients.

What is known already: Several waves of cyclic development of antral follicles within the same menstrual cycle have been demonstrated. Likewise, it has been shown that oocytes obtained from luteal phase ovarian stimulation (OS) have similar competence than those obtained in the follicular phase OS. Often, some patients require sequential OS in order to obtain more oocytes and increase their chances to reach embryo transfer. Thus, the DuoStim strategy could be an attractive alternative to reduce the time-to-pregnancy. However, prospective data and randomized trials that validate this strategy are lacking.

Study design, size, duration: We conducted a prospective, randomized controlled trial at our institution from[MCM1] [JAGV2] January 2017 to December 2020. A total of 80 poor prognosis patients aged over 38 years undergoing PGT-A were enrolled in two groups: 39 patients did two OS in consecutive cycles (control) whereas 41 women underwent two OS in the same menstrual cycle (DuoStim).

Participants/materials, setting, methods: Poor prognosis was defined as suboptimal responders. The primary outcome was the time needed to obtain an euploid embryo. The secondary outcomes were duration of stimulation, dose of gonadotropins, oocyte maturity rate, fertilization and blastocyst formation rates. Variables were expressed as mean ± standard deviation. Statistical analyses was performed by ANOVA and Chi-square tests, as appropriate. Differences were considered significant when *p-value* < 0.05.

Main results and the role of chance: The patients' baseline characteristics were similar between groups. We did not find any difference in the mean days of stimulation between the control and the DuoStim group (21.3 ± 1.6 *vs.* 23 ± 1.4, *p=0.105*), nor in the amount of gonadotropin required (4005 ± 450 *vs.* 4245 ± 430, *p=0.43*), number of MII oocyte (8.7 ± 1.8 *vs.* 6.8 ± 1.7, *p=0.159*), blastocyst rate (51.4% *vs.* 34.8%, *p=0.113*) and the number of euploid embryos (0.8 ± 0.4 *vs.* 0.6 ± 0.4, *p=0.45*). However, there was a significant difference in the average number of days until reaching an euploid blastocyst, favoring the DuoStim group (44.1 ± 2 *vs.* 23.3 ± 2.8, *p<0.001*). Comparing the follicular versus the luteal phase within the DuoStim group, the only difference detected concerns to the mean days of stimulation (10.3 ± 0.8 *vs.* 12.7 ± 0.9, *p<0.001*). We also observed a trend towards a higher fertilization (38.1% *vs.* 61.8%, *p=0.02*) and blastulation rate (23% *vs.* 53%, *p=0.03*) in the luteal phase of the DuoStim cycle.

Limitations, reasons for caution: The major limitation is related to the limited sample size, as it limits our power analysis (70%). On the other hand, it is one of the first randomized prospective pilot trial that compared the efficiency of performing two consecutive ovarian stimulation in different menstrual cycles with the DuoStim strategy.

Wider implications of the findings: This study clearly showed that the DuoStim protocol is not inferior to the conventional stimulation in terms of ovarian response and laboratory outcomes. Moreover, the DuoStim reduces the time to obtain an euploid embryo in poor prognosis patients, which is of great clinical utility.

Trial registration number: NCT03291821

O-112 Outcomes of random-start ovarian stimulation protocols as a possible evidence of the theory of antral follicles continuous recruitment

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Study question: We tried to validate the possibility and efficiency of ovarian stimulation (OS) started on any day of the menstrual cycle, based on a theory of continuous recruitment of antral follicles.

Summary answer: Formation of a pool of follicles with higher sensitivity to gonadotropic stimulation occurs several times during the menstrual cycle (MC).

What is known already: According to classical concepts and fundamental positions formulated in the middle of the last century, follicular recruitment occurs only once during the menstrual cycle - in the early follicular phase. Nowadays there is increasing evidence to suggest that there are multiple (two or three) antral follicular waves of recruitment during the MC. Also some researchers state that the process of follicle recruitment is continuous.

Study design, size, duration: This prospective clinical study was conducted at the V.I. Kulakov NMRC for OG&Pof Russia. The study included female cancer patients seeking retrieval and cryopreservation of oocytes and/or embryos before cancer treatment. 240 patients were selected for the study. The patients were divided into 5 groups depending on the cycle day on the moment when ovarian stimulation was initiated. All patients signed an informed consent form approved by the Ethics Committee.

Participants/materials, setting, methods: The 1st group consisted of patients who started standart OS from 1 to 5 days of the cycle (n=65); the 2nd - from 6 to 10 (n=36), the 3rd - from 11 to 15 (n=45), the 4th - from 16 to 22 (n=44), the 5th - from 23 to 28 (n=50). In the late follicular and luteal phase we performed OS without a pituitary modulator. The comparative analysis included features of oo-, embryogenesis and steroidogenesis.
Main results and the role of chance: The mean age, BMI and AMH were not different among groups. There were no LH rise or OHSS signs noticed in any groups, despite that OS in late follicular and luteal phase of the MC was performed with no GnRH antagonist addition.There was no statistically significant difference in the duration of stimulation, starting doses, total dose of FSH and HMG. The largest number of oocyte cumulus complexes was obtained in the 5th group (11 (9–21) vs 7 (3,5–15,5) in the 1st group, p=0,030). The greatest number of mature oocytes was obtained in the 4th and 1st groups. In the 2nd group the largest number of immature oocytes was obtained (37 (9.1%)). A smaller number of mature oocytes (165 (61.8%) vs 492 (72.9%) and 314 (77.5%), p = 0.001) was obtained in group 2 (compared with the 1st and the 4th groups), when stimulation was started in the presence of a dominant follicle . These periods coincided with higher estradiol and lower FSH serum levels. Based on our data the optimal moment for effective OS initiation starts with the decrease in serum estradiol which is approximately 48 hours before the menstrual bleeding.
Limitations, reasons for caution: The presented results could be applied mainly to young patients with high and normal ovarian reserve, who were in the main study group. In patients with low ovarian reserve, short menstrual cycle and early ovulation an issue of favorable time points for the initiation of OS should be resolved individually.
Wider implications of the findings: The data collected during our research could possibly contribute to future personification of OS protocols. Tailoring the ovarian stimulation protocols to the needs of the patients could decrease time needed for completing the protocol without affecting oocyte yield or their maturity.
Trial registration number: none

O-113 Effectiveness and treatment cost of assisted reproduction technology for women stimulated by gonadotropin in France: A cohort study using the National Health Database

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Study question: How effective is Assisted Reproduction Technology (ART) in terms of cumulative live birth rate (CLBR) in France, depending on the gonadotropin used?
Summary answer: Among 214,539 stimulations, originator follitropin-alfa was associated with significantly higher CLBR when compared to Highly Purified-Human Menopausal Gonadotropin (HP-HMG) and biosimilars.
What is known already: Deciding which type of gonadotropin to prescribe for a woman undergoing controlled ovarian stimulation (COS) remains difficult. The effectiveness of different gonadotropins is one factor to consider. However, studies comparing r-hFSH-alfa, its biosimilars and HP-HMG are scarce and are mostly based on a single ART treatment cycle and fresh embryo transfers. Some clinical trials have shown similar pregnancy, pregnancy loss, and live birth rates after fresh embryo transfer (ET) between HP-HMG and r-hFSH. However, because more oocytes are retrieved with r-hFSH when compared to HP-HMG, it is logical to hypothesize that the CLBR is higher with r-hFSH.
Study design, size, duration: A non-interventional study based on the French National Health System (SNDS) database was designed to assess the CLBR and treatment costs from the national payer perspective of four gonadotropin groups (originator follitropin-alfa (r-hFSH-alfa), its biosimilars, HP-HMG and r-hFSH-beta) used for COS cycles leading to oocyte pick-up (OPU) between 01/01/2013 and 31/12/2017 with a follow-up period up to 31/12/2018. The study compared CLBR, with originator r-hFSH-alfa as the reference.
Participants/materials, setting, methods: Women with COS cycles resulting in OPU with one of the specified gonadotropins were included. Data were extracted from billing and reimbursement records of outpatient healthcare consumption and national hospital discharge databases using a unique, anonymized patient number. CLBR was estimated using an Andersen–Gill model, adjusted for clinical baseline, stimulation and ET variables. Costs were reported as secondary outcomes.
Main results and the role of chance: 135,752 women (mean age 34.1), underwent 214,539 stimulations leading to OPU and contributed one (61.5%), two (24.8%), three (9.4%) or four (3.2%) COS cycles. COS cycles were stimulated with either Originator r-hFSH-alfa (46%), HP-HMG (29%), r-hFSH-beta (21%) or r-hFSH-alfa biosimilars (4%). Over the study period, CLBR reached 20.5%; 21.9% with originator r-hFSH-alfa, 17.9% with HP-HMG, 21.3% with r-hFSH-beta and 18.4% with r-hFSH-alfa biosimilars. After adjusting for age, pre-treatment, GnRH analog, ovulation triggering, luteal phase support, previous COS, fresh or frozen ET and type of center, as possible cofounding variables, the adjusted hazard ratio (HR) for CLBR (delivery [originator r-hFSH-alfa as reference]) was 0.88 (95% CI 0.86 to 0.95, p<0.0001) with HP-HMG; 0.98 (95% CI 0.95 to 1.00, p=0.1020) with r-hFSH-beta, and 0.84 (95% CI 0.79 to 0.90, p<0.0001) with r-hFSH-alfa biosimilars. Although the mean acquisition cost of r-hFSH-alfa during the study was 33% higher than HP-HMG and 20% higher than r-hFSH-alfa biosimilars, the global ART management costs were only 4% higher than HP-HMG, 3% higher than r-hFSH-beta, and similar to r-hFSH-alfa biosimilars.
Limitations, reasons for caution: Patients were included only from oocyte pick-up, due to missing data in the SNDS database, meaning that it was not possible to estimate the proportion of cancelled cycles. Furthermore, as r-hFSH-alfa biosimilars were only available since 2015, results for biosimilars should be interpreted with caution.
Wider implications of the findings: This population-wide French study confirms other Real-World and meta-analysis evidence that CLBR is higher with originator r-hFSH-alfa than with HP-HMG or r-hFSH-alfa biosimilars, respectively, and are relevant for healthcare professionals to support gonadotropin treatment decision making. To further support this, the cost analysis should be completed by a cost-effectiveness analysis.
Trial registration number: Not applicable

O-114 Improved safety and efficiency of individualised versus conventional gonadotropin dosing for ovarian stimulation in IVF/ICSI: an individual patient meta-analysis (IPD-MA)

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Study question: Does an individualised, weight- and AMH-based dosing approach with follitropin delta improve live birth rate, safety, and efficiency, compared to conventional dosing in IVF/ICSI?
Summary answer: Individualised ovarian stimulation performs similarly for live birth rate (increased in normal-high AMH), and reduces the incidence of OHSS and total FSH dosage.
What is known already: Previous studies investigated the effect of individualized gonadotropin dosing in IVF/ICSI using ovarian reserve tests such as anti-Müllerian hormone (AMH) and antral follicle count (AFC). A Cochrane Review concluded that individualised dosing in IVF is associated with a reduction of ovarian hyperstimulation syndrome (OHSS), but no effect on live birth rate. It is hypothesized that an individualised dosing approach is predominantly beneficial in the patients who are potentially normal or high responders. This study addresses the performance of a new human recombinant FSH (follitropin delta) with individualised dosing based on AMH and body weight.

Study design, size, duration: This is an individual participant data meta-analysis (IPD-MA) of three follitropin delta phase 3 trials, executed in Europe and North- and South America, South-East Asia, and Japan. All trials were randomized, controlled, assessor-blinded, multicenter studies in which individualised follitropin delta vs. conventional follitropin alpha or beta were compared. Women were followed from inclusion, at start of their first fresh IVF/ICSI cycle, until 4 weeks after live birth.
Participants/materials, setting, methods: Women aged 20-40 yrs, undergoing their first IVF/ICSI cycle, were randomly assigned to follitropin delta (AMH < 15 pmol/L: 12 µg/day; AMH ≥ 15 pmol/L: 0.10-0.19 µg/kg/day: maximum 12 µg/day) or conventional follitropin alpha or beta (150 IU/day for 5 days, possible subsequent dose adjustments). The IPD-MA was performed using logistic regression analysis. Planned subgroup analyses were performed for expected normal/ high responders (serum AMH ≥15 pmol/L), and expected low responders (serum AMH <15 pmol/L).
Main results and the role of chance: Nearly 2,700 women were randomised and exposed: n = 1,348 for conventional dosing regimen with follitropin alpha or beta, and n = 1,334 for individualised dosing with follitropin delta. Live birth rate was similar for both groups (29.5% in follitropin delta vs. 26.9% in follitropin alpha/beta; OR 1.14 (0.96-1.35)). However, in expected normal to high responders live birth rate was significantly increased for those receiving individualised follitropin delta (31.4% vs. 25.9%; OR 1.31 (1.06 - 1.62)). Mean number of transferred embryos/blastocysts was comparable (0.95 vs. 0.94, respectively; mean difference 0.0076; NS), and did not differ when subgroup analyses were performed for normal/high AMH and low AMH. The occurrence of early OHSS was significantly reduced in individualised follitropin delta (4.0% vs. 6.4%; OR 0.62 (95% CI 0.43-0.88)), in subgroup analyses a similar reduction was identified. Total dosage of FSH was significantly lower in individualized follitropin delta (84.5 vs. 112.1 µg; mean difference -27.5 µg (95% CI -30.0 - -25.1)), with a more pronounced effect in normal to high AMH (mean difference -36.5 µg (95% CI -39.2 - -33.7)). Gestational age and birth weight were similar. The IPD-MA identified similar findings among women from the three studies with their different ethnic backgrounds.
Limitations, reasons for caution: For individualised dosing with follitropin delta, it was observed that the number of cryopreserved embryos was significantly lower (2.4 vs. 3.0, mean difference -0.67 ($p < 0.05$)), and it remains unclear whether this affects cumulative live birth rate.
Wider implications of the findings: Individualised dosing with gonadotropin delta is similarly successful in terms of live birth (increased for normal-high AMH women), reduces safety risks, and is more effective with regard to gonadotropin dosage, compared with conventional dosing in IVF/ICSI. Treatment costs are reduced by prescription of lower gonadotropin doses and OHSS reduction.
Trial registration number: NCT01956110, NCT03228680, NCT03296527

SELECTED ORAL COMMUNICATIONS

SESSION 28: INVESTIGATING THE GENETIC BASIS OF REPRODUCTIVE PHENOTYPES

29 June 2021 Stream 4 10:00 - 11:30

O-115 Parental whole-exome sequencing allows the discovery of genetic causes of extreme IVF phenotypes such as oocyte/embryo developmental arrest and recurrent low fertilization

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Study question: Do whole-exome sequencing (WES) data from infertile women provide valuable information for the discovery of genes/pathways involved in extreme IVF phenotypes, i.e. oocyte/embryo developmental arrest?
Summary answer: The development of a specific bioinformatic WES pipeline revealed known and new candidate genes/pathways for isolated oocyte/embryo developmental failure,providing the foundation to scale up research.
What is known already: The use of *IVF* has made it possible to identify extreme and isolated infertility phenotypes such as recurrent low oocytes maturity (LMR), recurrent low fertilization rate (LFR), or preimplantation developmental arrest (PDA) that would remain concealed in natural conception attempts. Recent applications of WES in families with such extreme adverse IVF phenotypes have led to the discovery of new genes and pathways affecting unique functions of gametes and exclusive mechanisms necessary for early embryo development. Here, we apply a tailored bioinformatic approach to WES from women displaying extreme IVF phenotypes to discover new causative genes/pathways involved in unexplained infertility.
Study design, size, duration: Twenty-two infertile consanguineous women(December 2018-September 2020) suffering from long-term unexplained infertility. Eight cases were classified as PDA (<20% normally developed embryos in >2 IVF cycles), 8 as LMR (<20% mature oocytes in >2 IVF cycles), 4 as LFR (<20% of normally fertilized oocytes in >2 IVF cycles). Two women with recurrent IVF failure (>10 IVF cycles) were also included. A control set of 1660 WES from oocyte donors was used to control for false-positive discoveries.
Participants/materials, setting, methods: WES at 30X was performed on enrolled women's gDNA using Illumina short-reads technology. Following annotation, variants were filtered to prioritize putative detrimental variants in genes relevant for oocyte/embryonic development using a previously developed and validated pipeline that minimizes false-positive discoveries. Runs of homozygosity (ROH) within each sample were identified using Refined IBD software. Individual-level single-cell RNAseq (scRNAseq) dataset from 18 human oocytes was used to verify the expression of the identified target genes.
Main results and the role of chance: The variant prioritization pipeline employed identified 1,160 unique variants in 1,017 genes (average per sample 59.9 sd 8.5). 453 variants were private to this study compared to the 1000 Genomes and gnomAD databases, 3% affecting splicing and/or the gene product length. Significant 5-fold enrichment of 41 genes involved in DNA-damage and repair pathways commonly associated with ovarian function/oocyte quality was observed ($p<0.001$). TP53/AKT pathway also showed significant 5-fold enrichment for 45 genes ($p<0.001$). This finding is consistent with the known relationship between infertility and cell-cycle/cancer genes. Overall, 66.4% (675/1,017; 95%CI:63.4-69.3) of the targeted genes were expressed in MII human oocytes. Two women (9%) were homozygous carriers of missense pathogenic variants in known candidate genes previously associated to oocyte/embryo developmental arrest (TRIP13, chr5_901344_C/T, CADD percentile 0.999; PADI6, chr1_17394384_C/G, CADD percentile 0.999). Remarkably, four additional women were carriers of high-impact variants in JAKMIP1, a member of a recently characterized family of proteins involved in various cellular processes, including cytoskeleton rearrangement, cell polarization, and intracellular transport. High-impact JAKMP1 variants were never observed in the oocyte donor control dataset. JAKMIP1 mRNA was detected in each individual biological replicate of scRNAseq analysis of MII oocytes with a mean of 6 transcripts per million.
Limitations, reasons for caution: Functional analysis is ongoing to validate the newly identified genes, data need to be verified in different ethnicities. Nevertheless, this study demonstrates the establishment of a specific and scalable analytical framework that can be employed for the identification of genetic causes in unexplained infertility cases characterized by defective developmental patterns.
Wider implications of the findings: Scaling up this investigative approach would provide an effective strategy for discovering new genes/pathways in what is considered idiopathic infertility, further defining precision reproductive medicine interventions. Importantly, this study revealed lesions in genetic patterns involved in chronic diseases providing a molecular footprint of the well-established link between infertility and comorbidities.
Trial registration number: none

O-116 Genetic association analyses identify links between pelvic prolapse (PP) and connective tissue biology, cardiovascular and reproductive health.

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Study question: Can genome-wide association analysis unravel the biological underpinnings of PP and facilitate personalized risk assessment via genetic risk scores construction?
Summary answer: We unravel novel links with urogenital development and vascular health in PP and present polygenic risk score as a tool to stratify PP risk.
What is known already: Prolapse is characterized by a descent of the pelvic organs into the vaginal cavity. PP affects around 40% of women after menopause and is the main indication for major gynecological surgery, having an important health, social and economic burden. Although the etiology and biological mechanisms underlying PP remain poorly understood, prior studies suggest genetic factors might play a role. Recently, a genome-wide association study (GWAS) identified seven genome-wide significant loci, located in or near genes involved in connective tissue metabolism and estrogen exposure in the etiology of PP.
Study design, size, duration: We conducted a three-stage case-control genome-wide association study. Firstly, in the discovery phase, we meta-analyzed Icelandic, UK Biobank and the FinnGen R3 datasets, comprising a total of 20118 cases and 427426 controls of European ancestry. For replication we used an independent dataset from Estonian Biobank (7968 cases and 118895 controls). Finally, we conducted a joint meta-analysis, containing 28086 cases and 546321 controls, which is the largest GWAS of PP to date.
Participants/materials, setting, methods: We performed functional annotation on genetic variants unraveled by GWAS and integrated these with expression quantitative trait loci and chromatin interaction data. In addition, we looked at enrichment of association signal on gene-set, tissue and cell type level and analyzed associations with other phenotypes both on genetic and phenotypic level. Colocalisation analyses were conducted to help pinpoint causal genes. We further constructed polygenic risk scores to explore options for personalized risk assessment and prevention.
Main results and the role of chance: In the discovery phase, we identified 18 genetic loci and 20 genetic variants significantly associated with POP ($p<5\times10-8$) and 75% of the variants show nominal significance association ($p<0.05$) in the replication. Notably, the joint meta-analyses detected 20 genetic loci significantly associated with POP, from which 13 loci were novel. Novel genetic variants are located in or near genes involved in gestational duration and preterm birth (rs2687728 p=2.19x10-9, *EEFSEC*), cardiovascular health and pregnancy success (rs1247943 p=5.83x10-18, *KLF13*), endometriosis (rs12325192 p=3.72x10-18, *CRISPLD2*), urogenital tract development (rs7126322, p=4.35x10-15, *WT1* and rs42400, p=4.8x10-10, *ADAMTS16*) and regulation of the oxytocin receptor (rs2267372, p=4.49x10-13, *MAFF*). Further analyses demonstrated that POP GWAS signals colocalise with several eQTLS (including *EEFSEC, MAFF, KLF13*, etc.), providing further evidence for mapping associated genes. Tissue and cell enrichment analyses underlined the role of the urogenital system, muscle cells, myocytes and adipocytes ($p< 0.00001$, FDR<0.05). Furthermore, genetic correlation analyses supported a shared genetic background with gastrointestinal disorders, joint and musculoskeletal disorders and cardiovascular disease. Polygenic risk scores analyses included a total of 125551 people in the target dataset, with 5379 prevalent patients and 2517 incident patients. Analyzing the best GRS as a quintile showed association with incident disease (Harrell c-statistic= 0.603, SD=0.006).
Limitations, reasons for caution: This GWAS meta-analyses focused on European ancestry populations, which challenges the generalizability of GWAS findings to non-European populations. Moreover, this study included women with PP from population-based biobanks identified using the ICD-10 code N81, which limits analyses considering different disease stages and severity.
Wider implications of the findings: Our study provides genetic evidence to improve the current understanding of PP pathogenesis and serves as basis for further functional studies. Moreover, we provide a genetic tool for personalized risk stratification, which could help prevent PP development and improve the quality of a vast quantity of women.
Trial registration number: not applicable

O-117 Prevalence of Fragile X Mental Retardation 1 premutation (FMR1) in young infertile women with diminished ovarian reserve. Implications in clinical practice.

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Study question: Are young infertile patients with diminished ovarian reserve (DOR) elegible to perform the FMR1 premutation study?
Summary answer: Study of the *FMR1* premutation should be considered in infertile young patients with DOR in order to give them an adequate genetic counselling.
What is known already: *FMR1* gene may have some reproductive implications. Most notable is that FMR1 premutation expansions are associated with premature ovarian insufficiency (POI), diagnosed by amenorrhea or oligomenorrhea and FSH hormonal levels >25U/L before 40 years old. Presence of FMR1 premutation implies a risk of develop POI up to 24% and having an offspring with fragile X syndrome.

The frequency of *FMR1* premutation in general population is estimated in 0.3-0.7%. The role of *FMR1* premutation expansions in diminished ovarian reserve (DOR) patients is not clearly established and could be considered as a previous step to POI that may be related to sterility.
Study design, size, duration: Retrospective review of the *FMR1* gene study requested in patients of an Assisted Reproduction Unit of a tertiary Hospital in Barcelone from January-2016 to December-2019. A total of 307 cases were evaluated to determine the number of CGG repeat and AGG interruptions to assess the *FMR1* gene status.
Participants/materials, setting, methods: A total of 307 samples were assessed. Clinical and reproductive data were collected.

The *FMR1* status was requested on patients who present: a) POI (n=60); b) Family history of the FMR1 mutation (n=11); c) Infertile normo-ovulatory and young (≤35 years old) women with DOR defined as antral follicle count (AFC) < 7 and antimüllerian hormone <0.8ng/ml (n=71); d) Miscellaneous (n=29)

FMR1 was studied in 136 oocyte donors (screened by protocol), this was considered control group.
Main results and the role of chance: Mean age (±SD) of infertile DOR group was 32.7 +/- 2.1 years old (range 26-35) and showed altered ovarian reserve markers: AMH 0.43 ng/ml (SD ± 0.28) and AFC 4.27 (SD ± 2.1) follicles. In this group, 4 *FMR1* premutation cases were found.

Mean age (±SD) in control group was 26.28 +/- 5.2 years old and presented normal AMH and AFC values. One *FRM1* premutation carrier was detected among 136 patients, prevalence comparable to the non-sterile population.

The prevalence of FRM1 premutation was significantly higher in the DOR infertile group 5,6% vs 0,73% in the donors' group (p=0.02). Significant differences were observed also in terms of age and ovarian reserve markers between both groups.

Very few cases of POI patients or family history of Fragile X Syndrome have been evaluated, due to the fact we are not a reference of these kind of patients.

Among patients with a family history, 1 case from 11 (9.1%) was detected. In the POI group, three cases of premutation out of 60 (5%) were found.
Limitations, reasons for caution: This is a retrospective study with limited determinations of FMR1 studies. Donor screening and young infertile patients with significant low ovarian reserve are the main indications to request FMR1 status gene, so may lead to a selection bias.
Wider implications of the findings: These results should be confirmed prospectively in a higher population of infertile young patients with DOR, in order to identify the profile of infertile patient with diminished ovarian reserve who are elegible to perfom FMR1 gene premutation to give them an adequate clinical and genetic counselling.
Trial registration number: not apllicable

O-118 New insight into the genetic contribution of common variants to the development of extreme phenotypes of unexplained male infertility: a multicenter genome-wide association study

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Study question: What is the contribution of the common genetic variation to the development of unexplained male infertility due to severe spermatogenic failure (SPGF)?
Summary answer: Genetic polymorphisms of key immune and spermatogenesis loci are involved in the etiology of the most severe SPGF cases, defined by Sertoli cell-only (SCO) phenotype.
What is known already: Male infertility is a rising worldwide concern that affects millions of couples. Non-obstructive azoospermia (NOA) and severe oligospermia (SO) are two extreme manifestations characterized by SPGF. A genetic cause can be established in only around 20% of affected men, with the remaining cases being classified as otherwise unexplained. To date, the genome-wide association study (GWAS) strategy, although already successfully applied in several other complex traits and diseases, was less fruitful in studies that attempted to decipher the genetic component of unexplained SPGF, mainly due to both a lack of well-powered samples in different ancestries and limitations in study design.
Study design, size, duration: We designed a GWAS for unexplained male infertility due to SPGF including a total of 1,274 affected cases and 1,951 fertile controls from the Iberian Peninsula (Spain and Portugal) and Germany. Different biostatistics and bioinformatics approaches were used to evaluate the possible effect of single-nucleotide polymorphisms (SNPs) across the whole genome in the susceptibility to specific subtypes of unexplained SPGF.
Participants/materials, setting, methods: The case cohort comprised 502 SO and 772 NOA patients, who were subdivided according to histological phenotypes (SCO, maturation arrest, and hypospermatogenesis) and the outcome of testicular sperm extraction techniques (TESE) from testis biopsies. Genotyping was performed with the GSA platform (Illumina). After quality-control and genotype imputation, 6,539,982 SNPs remained for the analysis, which was performed by logistic regression models. The datasets went through a meta-analysis by the inverse variance weighted method under fixed effects.
Main results and the role of chance: Genetic associations with SCO at the genome-wide-level of significance were identified in the major histocompatibility (MHC) class II region (rs1136759, OR=1.80, P=1.32E-08) and in a regulatory region of chromosome 14 nearby the vaccinia-related kinase 1 (*VRK1*) gene (rs115054029, OR=3.14, P=4.37-08). VRK1 is a relevant proliferative factor for spermatogenesis that causes progressive loss of spermatogonia when disrupted in mouse models. The role of the MHC system in SCO susceptibility was comprehensively evaluated through a validated imputation method that infers classical MHC alleles and polymorphic amino acid positions. A serine at position 13 of the HLA-DRβ1 protein (defined by the risk allele of the lead variant rs1136759) explained most of the SCO association signals within the MHC class II region. This residue is located in the binding pocket of the HLA-DR molecule and interacts directly with the presented antigen. Interestingly, position 13 of HLA-DRβ1 is the most relevant risk amino acid position for a wide spectrum of immune-mediated disorders. The HLA-DRB1*13 haplotype (which includes the serine at position 13 and represents the strongest NOA-associated marker in Asians to date) was the strongest signal amongst the classical MHC alleles in our study cohort (OR=1.93, P=9.90E-07).
Limitations, reasons for caution: Although the statistical power for the overall analysis was appropriate, the subphenotype analyses performed had considerably lower counts, which may influence the identification of genetic variants conferring low to moderate risk effects. Independent studies in larger SCO study cohorts should be performed to confirm our findings.
Wider implications of the findings: The molecular mechanisms underlying unexplained SPGF are largely unknown. Our data suggest a relevant role of common genetic variation in the development of SCO, the most extreme histological phenotype of NOA. SCO is characterized by the loss of germ cells and, therefore, implies a considerably higher probability of unsuccessful TESE.
Trial registration number: N/A

O-119 Evaluating the reproductive potential of azoospermic men by germline mutation profiling

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Study question: Can whole exome sequencing (WES) of spermatozoa from azoospermic men identify mutations related to the etiology of their infertility and ability to support a pregnancy?
Summary answer: Key de novo germline mutations that affect sperm production and/or embryo developmental competence may explain reproductive failure in azoospermic men, regardless of the etiology.
What is known already: Azoospermia accounts for approximately 15% of male factor infertility cases. Although it can be caused by pre-testicular factors, the most recognized forms are testicular and post-testicular. While post-testicular azoospermia is mainly due to a mechanical obstruction, testicular azoospermia, the most severe form, is characterized by scattered functional germinal epithelia that strive to support the meiotic process during gamete development. To shed light on the etiology of this condition, genetic studies have been performed, albeit exclusively on peripheral blood. We chose to perform a genomic assessment of spermatozoa to preferentially detect germline mutations that may be passed to the progeny.
Study design, size, duration: In a 2-year period, we recruited infertile men undergoing epididymal aspiration for acquired obstructive azoospermia (OA; n=19) or testicular retrieval for nonobstructive azoospermia (NOA; n=10). Four additional men were included as fertile controls. Following WES, copy number variants (CNVs) and gene mutation profiles were compared between

the OA and NOA patients, and within those two categories, in relation to whether they generated a clinical pregnancy (fertile) or not (infertile).

Participants/materials, setting, methods: Spermatozoal DNA was extracted and amplified from the surgically retrieved specimens of consenting men (DNA concentration, 762±492 ng/ul; quality, 1.7±0.1 nm). CNVs, gene mutations, duplications, and deletions were detected using the CLC Genomic Server 9.0. Genes were considered duplicated or deleted when the read depth was >1.5 or <0.5 times the median read depth in the control. Common mutations in the OA and NOA cohorts were assessed according to the couples' clinical outcome.

Main results and the role of chance: Of 29 couples (maternal age, 41.9±7yrs; paternal age, 42.5±7yrs), 19 OA men underwent epididymal sperm retrieval (1.1±4x10^6/ml concentration, 9±12% motility) while 10 NOA men underwent testicular biopsy (0.03±0.2x10^6/ml concentration, 0.5±1% motility). WES did not reveal a significant difference in sperm aneuploidy between the two etiologies (OA, 1.8%; NOA, 1.9%).

In OA patients, only 3 genes were deleted, mainly housekeeping-related, while in the NOA cohort, 5 genes were deleted, involved in RNA transcription (POLR2L) and apoptosis (AP5M1), in addition to spermiogenic functions (AP1S2, AP1G2, APOE).

OA patients and their partners (maternal age, 36.8±4yrs) underwent 19 ICSI cycles that resulted in a pregnancy and delivery rate of 47.4% (9/19). Those able to reproduce (n=9) shared a mutation in ZNF749, a gene affecting only sperm production. The infertile individuals (n=10) all had a deletion on PRB1, controlling essential DNA replication.

NOA men and their partners (maternal age, 38.2±2yrs) underwent 10 ICSI cycles, yielding a clinical pregnancy rate of 70% (7/10). The fertile men (n=7) had a concurrent gene deletion involved in stem cell lineage differentiation (MPIG6B). Their infertile counterparts (n=3) had deleted genes involved in spermato/spermio-genesis (n=6) and, most importantly, in early embryonic development (MBD5, CCAR1, PMEPA1, POLK, REC9, REPIN1, MAPRE3, and ARL4C).

Limitations, reasons for caution: This is a novel study with limited observations. The presence of housekeeping-related mutations in fertile OA men as well as the DNA replication mutation in infertile OA patients, considering the acquired condition, remains puzzling. Although maternal age was controlled for, confounding factors related to the female partner cannot be excluded.

Wider implications of the findings: Screening men for germline mutations provides valuable information on their ability to reproduce, regardless of the etiology of azoospermia. Genome profiling was able to identify reasons for failed reproductive performance in azoospermic men, particularly those individuals with secretory azoospermia (NOA). Genomic profiling may identify gametes with retained embryo developmental competence.

Trial registration number: n/a

O-120 Unravelling reproductive failure by scrutinizing the male germline code

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Study question: Can whole exome sequencing (WES) of spermatozoal DNA provide insight into understanding the different steps that lead to inability of a couple to reproduce?

Summary answer: The identification of germline mutations can clarify different aspects of reproductive failure in couples with unexplained infertility.

What is known already: The limitation of a routine semen analysis in evaluating sperm characteristics, even according to the most stringent criteria, lies in its inability to provide substantial information on spermatozoa performance in ART. As a result, ancillary tests are being used to further assess the male gamete's reproductive potential. More recently, WES of the male genome, carried out on somatic cells, has become a powerful technique that can potentially shed light on the genetic causes of infertility. Here, we aim to preferentially detect germline mutations by sequencing spermatozoal DNA to pinpoint genes related to different underlying etiologies for reproductive failure.

Study design, size, duration: In a 5-year period, 25 couples subdivided according to their ICSI outcomes were included in this study. Sperm aneuploidy assessment by fluorescent in situ hybridization (FISH) and copy number variant (CNV) analysis by WES were carried out on ejaculated specimens from consenting male partners. Following CNV analysis, gene mutation profiles were compared between the fertile (n=10) and infertile cohorts (n=15), as well as in relation to the reasons for reproductive failure.

Participants/materials, setting, methods: FISH was performed on at least 1,000 sperm cells with a threshold of >1.6%. DNA was extracted and amplified from at least 500 spermatozoa (DNA concentration, 605±137 ng/ul; quality, 1.7±0.1 nm) for CNV analysis by WES. Mutations corresponding to the CNV were annotated and assessed using the CLC Genomic Server 9.0. Genes were considered duplicated or deleted when their read depth was >1.5 or <0.5 times the median read depth in the control.

Main results and the role of chance: Couples (n=25) (maternal age, 38.6±3yrs; paternal age, 39.7±5yrs) had normal somatic karyotypes with normal semen parameters (59.2±30x10^6/mL concentration, 44.8±18% motility) by WHO standards.

The fertile (n=10) cohort underwent 12 ICSI cycles, achieving an 82.6% (57/69) fertilization rate and 10/12 (83.3%) term pregnancies. The infertile cohort (n=15) underwent 21 ICSI cycles, achieving a 66% (62/84) fertilization rate and 5/17 (29.4%) clinical pregnancies, all resulting in pregnancy loss.

Sperm aneuploidy was consistently higher in the infertile (8.4%) versus fertile (4.0%) cohort (P<0.00001), as observed by FISH and DNA sequencing.

For both cohorts, WES detected deletions responsible for sperm–egg fusion (ADAM3A) and acrosomal development (SPACA, SPATA), explaining the necessity for ICSI in these couples.

The infertile cohort was characterized by 4 reasons for cycle failure: complete fertilization failure, poor embryo development, implantation failure, and pregnancy loss. Couples with complete fertilization failure (n=4) had deletions (PLCZ1, PIWIL1) indicating a sperm-related oocyte-activating deficiency. Those with poor embryo development (n=5) had mutations (HAUS1, KIF4A, XRN1) essential for centrosome integrity and spindle/microtubular stabilization. Couples who did not achieve pregnancy (n=7) had a mutation (IL9R) in common related to cytokine constituents in the implantation pathway. Those with pregnancy losses (n=5) had mutations (NLRP7, TP53) on post-implantation genes.

Limitations, reasons for caution: Several germline mutations, related to the different reasons for these couples' reproductive failure, were identified. Although intriguing, these findings are still new and need to be validated in a larger study population. In addition, while maternal age was controlled for, we cannot definitively exclude other confounding female factors.

Wider implications of the findings: Additional screening methods for infertile couples, particularly those with unexplained infertility, can be used to clarify elusive factors underlying their reproductive ability. A genetic screening of spermatozoal DNA may therefore be considered a potential tool in precision medicine for the treatment of subtle male factor infertility.

Trial registration number: n/a

INVITED SESSION

SESSION 29: THE ENIGMA OF ECTOPIC PREGNANCY

29 June 2021 Stream 1 11:45 - 12:45

O-031 Importance of blood flow to human implantation

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Abstract text

The success of embryo implantation depends on a plethora of factors, with embryo quality and endometrial receptivity belonging to the most important ones. The receptive phenotype of endometrium develops in reaction to appropriate estrogen stimulation in the proliferative phase and embryo-synchronized maturation warranted by the action of progesterone. Uterine blood supply, myometrial contractions and the activity of local immune cells also belong to important factors affecting the outcome of both natural and assisted reproduction. Endometrial perfusion was shown to be an independent receptivity parameter, showing a

direct association with pregnancy outcomes. Historically, the use of Doppler parameters of uterine vessels was studied as a reflection of blood flow to the endometrium. Although some authors showed a correlation between blood flow in uterine arteries and success rates in IVF cycles, it might not reflect the actual endometrial flow as most of the blood volume goes through myometrium, not endometrium. Currently, using available ultrasound tools – 2D/3D Power Doppler with VOCAL (Virtual Organ Computer-Aided Analysis) software enables clinicians to evaluate parameters of endometrial perfusion in a matter of minutes. In this method, ultrasound system can calculate indices reflecting endometrial blood flow - vascularity index (VI), endometrial flow index (FI), and endometrial vascularity flow index (VFI) which are based on the total and relative amounts of Power Doppler signal (corresponding to the blood flow) within the volume of interest. Endometrial blood flow parameters can be altered in implantation limiting conditions such as endometriosis or chronic intrauterine inflammation. It was also shown to be influenced by implantation-related hormones such as oxytocin.

Oxytocin receptor antagonists were shown to decrease uterine contractions in non-pregnant uteri of women being prepared for embryo transfer procedure. This class of medications has been extensively studied as potential candidates for medications promoting embryo implantation in IVF-ET treatments. In several studies, it was shown that mixed oxytocin/vasopressin V1A receptor antagonist atosiban, which is currently registered in Europe for the tocolysis in preterm labour, had the potential of improving implantation rates. Interestingly, this effect was confirmed also in women without pronounced contractions. Additionally, it has been demonstrated that oxytocin antagonism enhances endometrial decidualization and influences other parameters necessary for the acquisition of the endometrial receptivity phenotype. Considering that atosiban and other oxytocin antagonists relax uterine blood vessels and increase endometrial blood flow, it was hypothesized that improvement in endometrial perfusion could be an additional mechanism for observed support of embryo implantation.

A similar finding was confirmed in our study on nolasiban – a non-peptide, orally active, oxytocin receptor-specific antagonist. In the 1st phase study on volunteers undergoing estrogen/progesterone endometrial preparation reflecting the synchronization for embryo transfer, it was confirmed that application of nolasiban decreased uterine contractions and improved FI and VFI parameters of endometrial perfusion. Such an effect lasted for more than 24 hours after dosing. The study results suggested that oxytocin antagonism could have an effect on endometrial perfusion, and its potential clinical significance requires further investigation. In a longer perspective, once confirmed it would mean that, apart from the possibility of observation of endometrial blood flow, we could have a tool for improving it, which would hopefully lead to improved outcomes of assisted reproduction treatments.

O-032 The ESHRE GPR on ectopic pregnancy

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INVITED SESSION

SESSION 30: INVITED PATIENT SESSION: DEFINING ETHICAL LANDSCAPE OF TREATMENTS - PATIENTS VIEW

29 June 2021 Stream 3 11:45 - 12:45

O-033 Where ethics hits the patients needs - Case germany

K. Kordic[1]

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Abstract text

The German Embryo Protection Act was passed in 1990 and has never been changed since, other than minor changes in 2011 regarding PGD.

Germany is a country with 83 million inhabitants, where more than 6 million people of reproductive age are facing infertility or difficulties conceiving and delivering a healthy child. Germany also has the oldest and arguably most restrictive law regulating reproductive medicine, especially IVF. By passing a law with such an imposing title and starting the act with a list of prohibitions, 31 years ago German legislators made their intentions very clear: to protect the embryo rather than to help infertile people to become parents using assisted reproductive technology.

Since reproductive medicine has developed tremendously in the last 30 years and since the number of people in need of medical assistance to have a child is constantly growing,Germany now is not only far behind other European countries with its legislation but also seems to be against its patients and citizens in need of the assistance of modern medicine to have children.

How can we change an inappropriate and archaic law? How can we create a modern law which suits patients' needs without compromising ethics?

How can reproductive medicine in Germany come back on track again and serve the people by evidence based medicine in the first place?I

O-034 Infertility treatment law making in Europe: the clash of knowledge, ethics and business

A. Wilinska-Zelek[1]

[1]*Fertility Europe, Our Stork Association, Warszawa, Poland*

Abstract text

Infertility treatment law making in Europe: the clash of knowledge, ethics and business

Today, there is no common European set of rules for Assisted Reproduction Technology (ART). ART is now controlled by legislation in almost all European countries, substantial variations exist within the detail of that legislation.

Main legal differences between countries relate to: embryo selection, particularly by genetic screening, embryo freezing and embryo transfer, preimplantation genetic diagnosis (PGD), oocyte donation, anonymity of gamete donors, surrogacy, patient eligibility criteria (eg, sexual orientation, age), reimbursement and state funding.

The most complete survey ever of the ART legal and funding framework of 43 European countries was published in the ESHRE medical journal Human Reproduction Open: Calhaz-Jorge C, De Geyter C, Kupka MS, et al. Survey on ART and IUI: Legislation, regulation, funding and registries in European countries. Hum Reprod Open 2020; doi:10.1093/hropen/hoz044.

Unfortunately, changes of legislation are so dynamic that much of the information in this article is no longer up-to-date.

Lawyers observe that one of the most important rule of law "When the Law ceases to reflect the realities of Life, it is the Law that will Change" does not work in ART. In regard to this matter dominant rule is: "The Law will change only when it ceases to reflect the government's point of view and lobbyists' needs". Modern medical knowledge and the society's needs are often not the main concern during the law making discussion.

The speech discusses the issues related to infertility treatment law making in Europe with a focus of the problem that modern medical knowledge in this process is not taken into account at all. The author diagnoses numerous problem related to determining the border between medical knowledge, ethics and business in law making process.

The observed problems will be discussed on selected examples (from Poland, Greece and the United Kingdom) during presentation at the ESHRE on-line 37th Annual Meeting.

O-035 Searching for the perfect country - mapping out differences in fertility treatments and access to treatments across europe

K. Bye[1]

[1]*Ønskebarn, Norway, Bekkestua, Norway*

Abstract text

Fertility Europe has established a working group that is examining secondary data on legislation, regulation and funding of fertility treatment across Europe. The data has been mapped in order to easily visualize the "perfect country" in terms of fertility treatments[Vi1] . The map will be updated every two years in order to show progress towards more countries reaching the status of "perfect country". As the map provides a clear picture on the differences in access to treatments[Vi2] across Europe, it is expected to be a useful advocacy tool for

patient[Vi3] associations working to influence the current treatment situation in their country. This talk focuses on the work that has been done by the Fertility Europe working group to date, to create the map of the "perfect country", and the possibilities that such a map represents from a patient advocacy perspective.
treatmentS [Vi1] [Vi1]
treatmentS [Vi2] [Vi2]
patient associations' [Vi3] [Vi3]

INVITED SESSION

SESSION 31: COVID-19 AND ART: WHAT ARE TEH DATA AND HOW DO THEY AFFECT YOUR PRACTICE

29 June 2021 Stream 4 11:45 - 12:45

O-036 COVID-19, vertical transmission and ART pregnancy outcomes

B. Ata[1]

[1]*Koc University, Obstetrics & Gynecology, Istanbul, Turkey*

O-037 The impact of COVID-19 on male fertility: what do we know?"

A. Pacey[1]

[1]*University of Sheffield, Oncology and Metabolism, Sheffield, United Kingdom*

Abstract text

Since the very early days of the COVID-19 pandemic, concern has been raised about the possibility of damage to the male reproductive system in those men who are infected with SARS-CoV-2. This was first raised by the early observation (January 2020) that the route of infection was via the Angiotensin Converting Enzyme 2 (ACE-2) receptor present on host cells. ACE-2 was first described in the year 2000 and subsequently shown in 2004 to be selectively expressed in the Leydig cells of the adult testis. This opens up the possibility that although COVID-19 is primarily a respiratory infection, it may also infect the male reproductive system. The authors of several review articles have proposed that male fertility may be theoretically impacted by SARS-CoV-2 in a number of ways. This includes alteration of: (i) testicular architecture; (ii) reproductive hormone profiles (LH/FSH); (iii) spermatogenesis as evidenced by changes to ejaculate quality; (iv) sperm function (e.g., DNA damage); (v) sexual/erectile function; or a combination of all five. Clearly each of these individually may impact on the chance of pregnancy or live birth either in natural or medically assisted reproduction. There is also the possibility that SARS-CoV-2 may be transmitted sexually if sufficient numbers of SARS-CoV-2 are found in semen. Reassuringly, of 14 studies published to date, there is little evidence to suggest that SARS-CoV-2 is present in semen and so the possibility of sexual transmission in patient or donor samples can probably be discounted. However, there is currently an incomplete picture of whether semen quality is affected by SARS-CoV-2 infection as studies are often limited by the fact that no pre-infection control samples are available for direct comparison or they are too short to identify any long-term effects. Nevertheless, the few case-controlled studies published which compare semen quality infected with non-infected (control) individuals suggest that there may be a statistically significant alteration in sperm concentration and motility, although it is not clear whether this is linked to infection by the SARS-CoV-2 virus or simply a consequence of febrile illness and fever (or medication given to combat the infection). There is currently a lack of long-term data on any impact of COVID-19 on male reproductive hormones (although much speculation about the role that testosterone might play in the severity of disease). There are also emerging reports of increased risk of erectile dysfunction in men following SARS-CoV-2. In terms of birth rates, it is simply too early to tell whether these have been affected by the pandemic, given the possibility of lockdown affecting sexual behaviour in fertile couples and IVF clinic closures in infertile couples. In conclusion, although the COVID-19 pandemic has infected over 130 million people worldwide we still know too little about the impact of SARS-CoV-2 on the male reproductive system. Given the incidence of long-COVID, and the asymptomatic nature of the infection for some, it is important to commission and conduct long-term studies which can monitor the reproductive outcomes of young men who have survived a SARS-CoV-2 infection.

INVITED SESSION

SESSION 32: MHR SYMPOSIUM: MITOCHONDRIA: THE POWERHOUSE IN REPRODUCTION

29 June 2021 Stream 1 14:00 - 15:00

O-038 Mitochondrial DNA in the developing embryo

L. Cree[1]

[1]*University of Auckland, Obstetrics & Gynecology, Auckland, New Zealand*

O-039 Mitochondrial ATP generation in mammalian eggs and Ca2+ signalling

K. Swann[1]

[1]*Cardiff University, Dept. of Obstetrics and Gynaecology, Cardiff- Wales, United Kingdom*

Abstract text

In metaphase II arrested mammalian oocytes (eggs) and cleavage stage embryos the mitochondria are responsible for nearly all ATP production because glycolysis is inactivated. Luciferase assays show that ATP levels in eggs are strictly dependent upon pyruvate and fatty acid oxidation. The level of ATP in eggs appears to be maximal in conventional medium because the addition of extra mitochondrial substrates to eggs does not increase cytosolic ATP. The only clear elevation of ATP is seen at fertilization and is associated with sperm induced Ca2+ oscillations. Our recent findings suggest that the level of ATP modulates events at fertilization.

At fertilization, the egg is activated by sperm derived PLCzeta which triggers a series of Ca2+ oscillations, with each Ca2+ release event causes by inositol trisphosphate (InsP3). Previous studies have shown that mouse eggs are more sensitive to PLCzeta, and generate higher frequency Ca2+ oscillations, than human eggs. Mouse eggs also generate Ca2+ oscillations and activate in response to Sr2+ that directly stimulates InsP3 receptors. In contrast, human eggs that contain the same type of InsP3 receptors do not generate Ca2+ oscillations in response to Sr2+.

The difference in sensitivity of Ca2+ release between species can be explained by the fact that mouse eggs are about ten times more sensitive to InsP3 than human eggs. The reason for this difference appears to be due to ATP. The ATP level in unfertilized mouse eggs is about twice that in human eggs. Furthermore, the ability of mouse eggs to Sr2+ medium can be abolished by removing the mitochondrial substrate pyruvate, which reduces the ATP level. Adding back pyruvate to such eggs restores ATP levels promotes Sr2+ induced Ca2+ levels in mouse eggs. These data suggest that the level of ATP, possibly as ATP4-, modulates the sensitivity of the InsP3 receptor and the ability of eggs to generate Ca2+ oscillations. The level of cytosolic ATP may represent a significant 'egg factor' in determining the success of fertilization in humans. Enhancing mitochondrial ATP production could be useful in improving activation and embryo development after fertilization, or after artificial egg activation.

References:

Dumollard et al. (2009) Seminar in Cell and Developmental Biology 20, 346-353
Campbell and Swann (2006) Developmental Biology 298, 225-233
Storey et al. (2021) Molecular Human Reproduction 27, gaaa086

INVITED SESSION

SESSION 33: SEMEN ANALYSIS 2021: FROM THE ANCIENT TO THE MODERN TIMES?

29 June 2021 Stream 2 14:00 - 15:00

O-040 The evolution of WHO Manual. Where are we now?

S. Esteves[1,2,3]

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Abstract text

Male factor infertility is associated with impaired overall health, decreased life expectancy, lower quality of life and may affect reproductive outcomes even under assisted reproductive technology (ART) settings. Male factors, alone or combined with female factors, contribute to at least 50% of reported infertility cases. Despite this, the male partner is often overlooked in the evaluation and treatment of infertility. A routine semen analysis is frequently the only test carried out to assess a man's fertility potential.

The state-of-art on how the human semen should be assessed is provided by the World Health Organization (WHO), which periodically releases manuals that include specific protocols and reference standards. These manuals include detailed laboratory methods for semen examination, protocols for sperm preparation and cryopreservation, quality assurance and quality control, results' interpretation, and reference ranges. Unlike the previous four versions, the latest 2010 WHO reference values relied on clinical chemistry principles to generate 95% intervals for sperm volume, count, motility, vitality, and morphology from recent fathers. The fifth centile was deemed suitable for representing semen characteristics at lower limits. The reference values ultimately obtained were markedly lower than those previously reported, raising concerns about its clinical utility and generalizability. Criticisms included the limited geographical area of patients analyzed, the methods used for semen evaluation, and the potential impact of the new reference range on patient referral, diagnosis, and treatment guidance. An updated new WHO manual (6th edition) is about to be released with much expectation.

Although semen analysis remains one of the cornerstones of the infertility evaluation, a male infertility workup primarily based on routine semen analysis does not provide men with an optimal fertility pathway for many reasons. First, reference intervals do not reliably distinguish fertile from subfertile subjects. Second, an individual patient's results have limited prognostic value for both natural and assisted conception unless at extreme lower limits. Third, there is a wide variation in how laboratories perform a semen analysis. Lastly, routine semen analysis does not detect sperm DNA defects that might adversely impact embryo development, implantation, and offspring's health.

Guidelines issued by professional societies recommend that a full andrological assessment be performed in all men with couple infertility. Well-trained reproductive urologists or clinical andrologists should perform the male evaluation, including a detailed history, physical examination, semen analysis, endocrine assessment, and other tests as needed. Therefore, the importance of WHO manuals remains critical. However, the goals of a comprehensive male infertility workup go beyond the laboratory assessment of human semen. It comprises i. *Diagnosis*, i.e., detection of any underlying relevant medical or lifestyle conditions potentially impairing the (reproductive) health of the male or his offspring; ii. *Counselling*, particularly regarding the impact of infertility, genetic factors, age, and lifestyle on pregnancy prospects, reproductive and overall health, and offspring's well-being; and iii. *Management Guidance*, i.e., identifying optimal treatment options to improve the likelihood of achieving natural pregnancy or ART success. The prevention and management of male infertility are integral components of comprehensive sexual and reproductive health services needed to attain a sustainable development goal.

O-041 Functional sperm diagnostics: The evidence behind commercially available tests

J. Hotaling[1]

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Abstract text

Despite the fact that the semen analysis has remained the cornerstone of the male infertility evaluation for decades, it has significant limitations. Among them are the inability to completely predict reproductive potential and the inability to analyze the fitness of individual sperm. There remains a real need for novel tests that can determine which interventions for male infertility are the most likely to result in a significant improvement in reproductive potential, to analyze the chance of success or failure with ART and to determine if there exists a sub population of sperm whose use could lead to optimal chances of success with fertility treatments. In this talk we will cover what the limitations of the current semen analysis are, what novel tests are available and delve into the impact of future technologies such as microfluidics, machine learning and others to optimize the diagnostic armamentarium of male fertility assessment. We will also focus on tools that could, potentially, non-invasively image sperm and report reproductive fitness while maintaining viability for ART.

Trial registration number:
Study funding:
Funding source:

INVITED SESSION

SESSION 34: EUROPEAN AND GLOBAL ART MONITORING

29 June 2021 Stream 3 14:00 - 15:00

O-042 Assisted Reproductive Technology (ART) in Europe 2018 and development of a strategy of vigilance. preliminary results generated from european registers by ESHRE

C. Wyns[1], **Ch. De Geyter**[2], **C. Calhaz-Jorge**[3], **MS. Kupka**[4], **T. Motrenko**[5], **J. Smeenk**[6], **C. Bergh**[7], **A. Tandler-Schneider**[8,1] **A. Rugescu**[9], **S. Vidakovic**[10], **V. Goossens**[11]

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[3]*Faculdade de Medicina da Universidade de Lisboa, Portugal.*
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[6]*Elisabeth Twee Steden Ziekenhuis, Tilburg, the Netherlands.*
[7]*Dept of Obstetrics and Gynecology, Inst of Clinical Sciences, Göteborg University, Göteborg, Sweden.*
[8]*Fertility Center Berlin, Berlin, Germany.*
[9]*National Transplant Agency, Romania.*
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[11]*ESHRE Central Office, Meerstraat 60, Grimbergen, Belgium.*

Study question: What are the reported data on cycles in ART, IUI and fertility preservation interventions in 2018 as compared to previous years, as well as the main trends over the years?

Summary answer: AUTHOR: The 22th ESHRE report on ART and IUI shows a progressive increase in reported treatment cycle numbers in Europe, a small decrease in the number of transfers (IVF + ICSI) with more than one embryo with a trend to decreasing multiple delivery rates, higher pregnancy and delivery rates after FER compared to fresh IVF and ICSI cycles, and outcomes for IUI cycles similar to previous years.

What is known already: Since 1997, ART aggregated data generated by national registries, clinics or professional societies have been collected, analysed by the European IVF-monitoring Consortium (EIM) and reported in 21 manuscripts published in Human Reproduction and Human Reproduction Open.

Study design, size, duration: Yearly collection of European medically assisted reproduction (MAR) data by EIM for ESHRE. The data on treatments performed between January 1 and December 31 2018 in 34 European countries were provided by either National Registries or registries based on personal initiatives of medical associations and scientific organisations.

Participants/materials, setting, methods: In all, 1004 clinics offering ART services in 34 countries reported a total of 827 545 treatment cycles, involving 132 332 with IVF, 342 589 with ICSI, 260 013 with frozen embryo replacement (FER), 44 854 with preimplantation genetic testing (PGT), 42 869 with egg donation (ED), 406 with IVM of oocytes and 4482 cycles with frozen oocyte replacement (FOR). European data on IUI using husband/partner's semen (IUI-H) and donor semen (IUI-D) were reported from 783 institutions offering IUI

in 24 and 20 countries, respectively. A total of 132 624 treatments with IUI-H and 43 140 treatments with IUI-D were included. A total of 12 609 fertility preservation (FP) interventions from 13 countries including oocyte, ovarian tissue, semen and testicular tissue banking in pre-and postpubertal patients were reported.
Main results and the role of chance: In total, 1004 IVF clinics participated (93.4% of registered clinics in the participating countries). Next to these also 783 IUI units reported their data. In the 34 reporting countries, after IVF the clinical pregnancy rates (PR) per aspiration and per transfer in 2018 were similar to those observed in 2017 (28.7% and 41.6% versus 29.4% and 39.0%, respectively). After ICSI the corresponding rates were also similar to those achieved in 2017 (26.3% en 40.9% versus 27.3% and 40.2%). After FER with own embryos the PR per thawing is still on the rise, from 30.2% in 2017 to 33.0% in 2018. After ED the PR per fresh embryo transfer was 49.8% (49.2% in 2017) and per FOR 39.6% (43.3% in 2017). In IVF and ICSI together, the trend towards the transfer of fewer embryos continues with the transfer of 1, 2, 3 and ≥4 embryos in 51.1%, 45.4%, 3.4% and 0.1% of all treatments, respectively (corresponding to 46.0%, 49.2%, 4.5% and 0.3% in 2017). This resulted in a proportion of singleton, twin and triplet DRs of 86.9%, 12.8% and 0.3%, respectively (compared to 85.5%, 14.2% and 0.3%, respectively in 2017). Treatments with FER in 2017 resulted in twin and triplet DR of 9.3% and 0.1%, respectively (versus 11.2% and 0.2% in 2017). After IUI, the DRs remained similar at 9.1% after IUI-H (8.9% in 2017) and at 12.3% after IUI-D (12.4% in 2017). Twin and triplet DRs after IUI-H were 8.4% and 0.3%, respectively (in 2017: 8.1% and 0.3%) and 6.7% and 0.2% after IUI-D (in 2017: 6.9% and 0.2%). The majority of FP interventions included the cryopreservation of ejaculated sperm (n=8 257 from 13 countries) and of oocytes (n=3230 from 13 countries).
Limitations, reasons for caution: As the methods of data collection and levels of completeness of reported data vary among European countries, the results should be interpreted with caution. For this abstract a number of countries was not able to provide adequate data about the number of centers and initiated cycles and deliveries.
Wider implications of the findings: The 22nd ESHRE report on ART and IUI shows a continuous increase of reported treatment numbers and MAR-derived livebirths in Europe. Being already the largest data collection on MAR in Europe, continuous efforts to stimulate data collection and reporting strive for future quality control and completeness of the data and offer higher transparency and vigilance in the field of reproductive medicine.
Trial registration number:
Study funding:
Funding source:

O-043 International committee for monitoring assisted reproductive technologies (ICMART) preliminary world report on ART, 2017

G.D. Adamson[1]

[1]*Equal3 Fertility, Reproductive Endocrinology and Infertility, Cupertino, CA U.S.A*

O-044 Chinese Society of Reproductive Medicine 2018 annual report on ART

C. Deng[1]

[1]*Peking Union Medical College Hospital, IVF Center, Beijing, China*

Abstract text
Objective: To analysis the Chinese ART data in 2018 to provide evidence for utilization of various ART.
Methods: The data of ART of 263 Reproductive Centers in the mainland of China in 2018 were collected by CSRM ART Data Reporting System. A cross-sectional survey of the use of ART technology was performed.
Results: In 2018, the CSRM data reporting system reported 105 610 AID/AIH cycles, 323 938 oocyte retrieval cycles, 147 129 fresh embryo transfer cycles, 254 012 frozen-thawed embryo transfer (FET) cycles, and 204 688 newborn . The patient's age was mainly concentrated in the group <35 years old, accounting for 63.75%. The pregnancy rate and live birth rate of retrieval cycles were 52.49% and 42.23% respectively. The pregnancy rate and live birth rate of FET cycles were 48.71% and 37.68% respectively. Among ART complications, the incidence of moderate to severe OHSS was 1.42%, 0.03% postoperative bleeding, 0.01% postoperative pelvic infection, and 0.04% the other complications. The incidence of birth defects of IVF was 0.87%.
Conclusions: This study uses "CSRM data reporting system" data to describe and analyze the current status of ART, basically consistent with the comparison in 2016 and 2017 that most cycles with good outcomes. However, no clear conclusions have been drawn on the changes of PGD/PGS cycle, all-freeze cycle and comprehensive analysis should be conducted by combining with laboratory data.
Trial registration number:
Study funding:
Funding source:

INVITED SESSION

SESSION 35: MODELLING HUMAN REPRODUCTION: HOW FAR ARE WE?

29 June 2021 Stream 4 14:00 - 15:00

O-045 Synthetic human embryo-like structures: a new paradigm for human embryology

J. Fu[1]

[1]*University of Michigan- Ann Arbor, Mechanical Engineering- Biomedical Engineering- Cell and Developmental Biology, Ann Arbor, U.S.A.*

Abstract text
Early human development remains mysterious and very difficult to study. Recent advances in mammalian embryology, stem cell biology, organoid technology, and bioengineering have contributed to a significant interest in bottom-up, synthetic stem cell-derived models of human development (or embryoids). The controllability and reproducibility of human embryoids coupled with the ease of genetically modifying stem cell lines, the ability to manipulate culture conditions and the simplicity of live imaging make them robust and attractive systems to disentangle cellular behaviors and signaling interactions that drive human embryogenesis. In this talk, I will describe our effort in using human pluripotent stem cells (hPSCs) to develop tractable experimental models of the peri-implantation embryonic development and neurulation. The peri-implantation human embryoids developed by us recapitulate key early post-implantation developmental landmarks successively, including pro-amniotic cavity formation, amniotic ectoderm-epiblast patterning, primordial germ cell specification, and development of the primitive streak with controlled anteroposterior polarity. I will further discuss an hPSC-based neuroectoderm patterning model to recapitulate the formation of the neural plate and another patterned neural tube model with fully defined anterior-posterior and dorsal-ventral axes.

O-046 Implantation and beyond, the endometrial perspective

J. Aplin[1]

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Abstract text
Early pregnancy failures are spread throughout the first few weeks, suggesting that impairment of normal biological processes can occur at sequential stages: epithelial attachment and breaching, early stromal and then decidual invasion, glandular and vascular invasion. Biopsy-based transcriptomics and proteomics have failed to demonstrate a reproducible or interpretable molecular signature for endometrial receptivity, but single cell RNAseq suggests a step change in the epithelial transcriptome in the mid secretory phase, consistent with the appearance of new cell phenotype(s). Previously unseen heterogeneity in both epithelial and stromal cell populations has become evident, but gene signatures have not yet achieved a level of resolution that allows insights clear enough to decode the biology of the receptive state. However, methodology for propagating and recombining endometrial cell populations into 3D engineered tissue models has advanced, so that new mechanistic questions can be asked. Initial acquisition of receptivity to implantation is followed by the development of a

supportive environment for embryos that possess the capacity to progress, with maternal cell populations (epithelial, stromal, immune and vascular) acting cooperatively within a remodelling extracellular matrix (ECM). A balance must be achieved in the ECM between breakdown, with opening of hydrated spaces to allow expansion of the embryonic sac while maintaining a substrate for stable physical attachment to allow invasion by extravillous trophoblast. The extent and nature of cellular trafficking to and from the uterus is important before and during early pregnancy. There is evidence that regulation of a resident senescent cell subpopulation by uterine NK cells occurs in concert with ECM remodelling to achieve a functionally supportive environment in the early first trimester. Access of bone marrow-derived cells to vessel walls is regulated by placental-endothelial signalling to initiate remodelling for the increased blood flow to the conceptus that is required in later pregnancy. Thus 'receptivity' and 'supportiveness' require normal cell proportions and functional phenotypes in multiple endometrial cell populations and their physical environment in order to allow a well-calibrated sequential developmental response in the conceptus.

SELECTED ORAL COMMUNICATIONS
SESSION 36: AUTOMATION IN THE IVF LAB
29 June 2021 Stream 1 15:15 - 16:30

O-121 Exploring non-invasive methods to predict Ploidy Status: Combination of blastocyst morphology image analysis and proteomic profiles by using Artificial Neural Networks

A. Garg[1], L. Bari[1], M.A. Valera[1], E.I. Fernandez[2], J.C. Rocha[2], A. Quiñonero[3], F. Domínguez[3], M. Meseguer[4]

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[3]IVIRMA Foundation, Innovation, Valencia, Spain ;
[4]IVIRMA, IVF laboratory, Valencia, Spain

Study question: Is the blastocyst morphology image analysis combined with the protein content of spent embryo culture medium a suitable way to predict embryo ploidy?
Summary answer: Morphological variables from blastocyst image analysis combined with IL-6 or MMP-1 concentration in spent culture medium showed more than 80% of accuracy for euploidy prediction.
What is known already: An artificial intelligence model based on the proteomic profile of euploid embryos and morphological data from blastocyst time-lapse images has been recently published (Bori et al., 2020). The most promising artificial neural network (ANN) algorithm considered 20 morphological variables extracted from image analysis and two proteins detected in embryo culture medium (MMP-1 and IL-6). The overall success rate on blind test data was 72.7% for live birth prediction. The main aim of the present study was to check if the same morphological variables combined with MMP-1 or IL-6 with a cost-effective technique could discriminate between euploid and aneuploid embryos.
Study design, size, duration: This prospective study included 120 embryos from the preimplantation genetic testing for aneuploidies (PGT-A) program. A single blastocyst image was obtained for each embryo and their spent culture medium was collected on the day 5/6 of embryo development (day of trophectoderm biopsy). Morphological variables were extracted for all the blastocyst. On the other hand, we quantified IL-6 levels of 67 embryos and MMP-1 levels of 53 embryos. Resulting parameters were used to predict PGT-A results.
Participants/materials, setting, methods: Blastocyst images were imported into Matlab software and segmented into regions of interest. We obtained 20 mathematical variables related to measurements of areas, number of pixels and texture analysis. Chromosome analysis was performed using next-generation sequence technology. In parallel, 20 µL of spent culture medium from each blastocyst was analyzed with ELISA kits (IL-6 or MMP-1). Protein concentrations and morphological variables were used as input data for an ANN associated with genetic algorithms.
Main results and the role of chance: The euploid rate for the set of embryos included in the IL-6 group was 51.4%. The ANN was trained with 49 embryos and blind tested with 18 embryos. Following results correspond to euploidy prediction on the blind test. The sensitivity, specificity, accuracy and area under the ROC curve (AUC) were: 0.56, 0.78, 0.67 and 0.72 considering only IL-6 values; 0.88, 0.78, 0.83 and 0.61 considering IL-6 values and blastocyst morphological data extracted from the image analysis. The euploid rate for the set of embryos included in the MMP-1 group was 51.9%. The ANN was trained with 39 embryos and blind tested with 14 embryos. Following results correspond to euploidy prediction on the blind test. The sensitivity, specificity, accuracy and AUC were: 0.71, 0.57, 0.64 and 0.67 considering only MMP-1 values; 0.86, 0.86, 0.86 and 0.61 considering MMP-1 values and morphological data extracted from the image analysis.
Limitations, reasons for caution: The detection limit in protein quantification is the main limitation of our study. The small number of embryos and the specific culture medium used should be considered for the model application.
Wider implications of the findings: Our preliminary results showed that blastocyst morphology and embryo secretomics could be useful for euploidy prediction by using artificial intelligence techniques. These findings may contribute to the emerging era of non-invasive preimplantation genetic testing (ni-PGT-A).
Trial registration number: not applicable

O-122 ICSI in a box: development of a successful automated sperm injection robot with external supervision and minimal manual intervention.

N. Costa-Borges[1], G. Giralt[2], E. Albó[2], A. Alvarez[3], J. Ramos[3], I. Hernandez[3], M. Luis[2], G. Calderón[1], S. Munne[2]

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[2]Overture, Research and Development, Barcelona, Spain ;
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Study question: Is it possible to automate the way sperm is injected in an oocyte and improve ICSI consistency between embryologists?
Summary answer: The developed ICSI robot demonstrated a high degree of consistency and operator skill independence, allowing human supervision and external control, but minimal manual intervention.
What is known already: ICSI is a clinical procedure that is currently performed worldwide in most IVF centers and its use will only increase with more utilization of egg freezing. Since its implementation, the technique has been conducted manually by highly skilled embryologists. However, success rates can vary significantly depending on the experience of the operator. We leverage our experience in robotics, AI algorithms and embryology to develop an automated ICSI robot that requires minimal manual intervention with the aim to standardize the consistency of the procedure and, ultimately, improve overall results maintaining embryologist oversight.
Study design, size, duration: The ICSI robot was developed to have supervised automated control on critical steps of the injection procedure, including injection pipette advancement, zona pellucida and oolemma penetration with piezo-pulses, and pipette removal after injection. Manual intervention is required only for immobilization and capture of spermatozoa with a joystick gamepad and to release the sperm in the ooplasm, without the need for micromanipulation skills. In parallel, piezo-ICSI was performed in a conventional micromanipulation station as a control.
Participants/materials, setting, methods: Hamster and mouse oocytes were collected from superovulated females. For testing the efficiency of the automated system, hamster oocytes were injected with human donor sperm, as historically used in manual ICSI training programs, and survival rates evaluated after overnight culture. Mouse oocytes were injected with mouse sperm heads and subsequently cultured *in vitro* for five days. Blastocysts obtained were vitrified and embryo transfers are ongoing to evaluate term developmental rates.
Main results and the role of chance: The technical components of the ICSI robot were engineered to integrate AI algorithms, optics, cell microinjectors and mechatronics. AI algorithms were developed to identify the morphological structures of MII oocytes, including the zona pellucida, perivitelline space and polar body, both in the hamster and mouse models. The system detects and analyzes both the pipette and the oocyte and chooses the best area and plane for injection, allowing automated control of the subsequent injection steps. Using the hamster oocyte-human sperm model, a survival rate of 91% (n=110) was

achieved with the robot, which was statistically similar (p=0.335) to the results obtained in the controls injected manually (96%, n=28). The average time spent in each injection cycle, which includes scanning of the oocyte and injection pipette, and injection of the sperm into the oocyte, was approximately two minutes per ICSI operation. This time was comparable to the time required by highly experienced operators with manual piezo-injection. In the mouse, 91% (n=53) of the oocytes injected survived the procedure, of which, 92% developed to two-cells and 87% to the blastocyst stage. No statistical differences were found when compared these efficiencies with manual controls (n=40, 98%, 97% and 92%, respectively).

Limitations, reasons for caution: The developed ICSI robot has shown highly consistent results, independently of operator skills, both in hamster and mouse oocytes. However, additional validations should be performed to enlarge the sample size of injected oocytes and to evaluate the efficiency of the system in other oocyte species, including translational studies to humans.

Wider implications of the findings: The combination of multidisciplinary teams allows the development of automated processes that can reduce variability in certain IVF procedures, while supervised and assisted by experienced embryologists. It is expected that other laboratory procedures can be automated in the field of assisted reproductive treatments in a near future.

Trial registration number: N/A

O-123 Calibration of artificial intelligence (AI) models is necessary to reflect actual implantation probabilities with image-based embryo selection

M.F. Kragh[1], J.T. Lassen[2], J. Rimestad[2], J. Berntsen[2]

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Study question: Do AI models for embryo selection provide actual implantation probabilities that generalise across clinics and patient demographics?

Summary answer: AI models need to be calibrated on representative data before providing reasonable agreements between predicted scores and actual implantation probabilities.

What is known already: AI models have been shown to perform well at discriminating embryos according to implantation likelihood, measured by area under curve (AUC). However, discrimination performance does not relate to how models perform with regards to predicting actual implantation likelihood, especially across clinics and patient demographics. In general, prediction models must be calibrated on representative data to provide meaningful probabilities. Calibration can be evaluated and summarised by "expected calibration error" (ECE) on score deciles and tested for significant lack of calibration using Hosmer-Lemeshow goodness-of-fit. ECE describes the average deviation between predicted probabilities and observed implantation rates and is 0 for perfect calibration.

Study design, size, duration: Time-lapse embryo videos from 18 clinics were used to develop AI models for prediction of fetal heartbeat (FHB). Model generalisation was evaluated on clinic hold-out models for the three largest clinics. Calibration curves were used to evaluate the agreement between AI-predicted scores and observed FHB outcome and summarised by ECE. Models were evaluated 1) without calibration, 2) calibration (Platt scaling) on other clinics' data, and 3) calibration on the clinic's own data (30%/70% for calibration/evaluation).

Participants/materials, setting, methods: A previously described AI algorithm, iDAScore, based on 115,842 time-lapse sequences of embryos, including 14,644 transferred embryos with known implantation data (KID), was used as foundation for training hold-out AI models for the three largest clinics (n=2,829;2,673;1,327 KID embryos), such that their data were not included during model training. ECEs across the three clinics (mean±SD) were compared for models with/without calibration using KID embryos only, both overall and within subgroups of patient age (<36,36-40,>40 years).

Main results and the role of chance: The AUC across the three clinics was 0.675±0.041 (mean±SD) and unaffected by calibration. Without calibration, overall ECE was 0.223±0.057, indicating weak agreements between scores and actual implantation rates. With calibration on other clinics' data, overall ECE was 0.040±0.013, indicating considerable improvements with moderate clinical variation.

As implantation probabilities are both affected by clinical practice and patient demographics, subgroup analysis was conducted on patient age (<36,36-40,>40 years). With calibration on other clinics' data, age-group ECEs were (0.129±0.055 vs. 0.078±0.033 vs. 0.072±0.015). These calibration errors were thus larger than the overall average ECE of 0.040, indicating poor generalisation across age. Including age as input to the calibration, age-group ECEs were (0.088±0.042 vs. 0.075±0.046 vs. 0.051±0.025), indicating improved agreements between scores and implantation rates across both clinics and age groups. With calibration including age on the clinic's own data, however, the best calibrations were obtained with ECEs (0.060±0.017 vs. 0.040±0.010 vs. 0.039±0.009). The results indicate that both clinical practice and patient demographics influence calibration and thus ideally should be adjusted for.

Testing lack of calibration using Hosmer-Lemeshow goodness-of-fit, only one age-group from one clinic appeared miscalibrated (P=0.02), whereas all other age-groups from the three clinics were appropriately calibrated (P>0.10).

Limitations, reasons for caution: In this study, AI model calibration was conducted based on clinic and age. Other patient metadata such as BMI and patient diagnosis may be relevant to calibrate as well. However, for both calibration and evaluation on the clinic's own data, a substantiate amount of data for each subgroup is needed.

Wider implications of the findings: With calibrated scores, AI models can predict actual implantation likelihood for each embryo. Probability estimates are a strong tool for patient communication and clinical decisions such as deciding when to discard/freeze embryos. Model calibration may thus be the next step in improving clinical outcome and shortening time to live birth.

Trial registration number: This work is partly funded by the Innovation Fund Denmark (IFD) under File No. 7039-00068B and partly funded by Vitrolife A/S

O-124 Contact-free oocyte denudation in a chip-scale ultrasonic microfluidic device

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Study question: To design and test an automated microfluidic device to revolutionize the cumulus-oocyte-complex (COC) denudation procedure for intracytoplasmic sperm injection (ICSI) using murine oocytes.

Summary answer: Oocyte exposure to temperature variation, mechanical stress, and prolonged chemical treatment during denudation was mitigated using our microfluidic device based on surface acoustic waves (SAWs).

What is known already: COC denudation is a prerequisite for many ART procedures such as ICSI. However, this procedure is based on manual pipetting (MP), which lacks standardization and requires experienced embryologists to perform. Inadequate MP may damage oocytes through prolonged enzymatic treatment or high fluidic stresses and may jeopardize gamete competence. The use of microfluidic devices based on porous membranes or microchannels has been adopted by many laboratories for sperm selection. Of these, microchannel devices may also be adapted for denudation with minimal mechanical stress in a controlled microenvironment. However, oocyte manipulation and extraction have proven difficult to achieve.

Study design, size, duration: We developed a novel ultrasonic microfluidic device based on a microwell design manufactured with Polydimethylsiloxane (PDMS). The SAWs were generated by 4 interdigitated transducers (IDTs) arranged in an orthogonally symmetric pattern. A non-toxic dosage of ultrasonic waves, similar to those used in gynecology and obstetrics, was applied. COCs were denuded by induced acoustic streaming and acoustic radiation force. Denudation rate, embryo development, and pregnancy outcomes were assessed and compared to control oocytes denuded by MP.

Participants/materials, setting, methods: For each run, up to 10 individual COCs from super-ovulated B6D2F1 mice were loaded into the microwell alongside diluted hyaluronidase (20 IU/ml) and denuded by 80 or 200 MHz SAWs. Denuded oocytes were fertilized by piezo-actuated ICSI using spermatozoa from the same strain. Pre-implantation embryo development was assessed in a time-lapse incubator for up to 96 h. High-quality blastocysts were transferred to 2.5-dpc pseudo-pregnant CD-1 surrogates. Pregnancy and offspring health were observed.

Main results and the role of chance: Using alternating frequency sweep in a pulse-repetition mode, we swirled the fluid inside the microwell consistently and tumbled COCs inside the microwell to expose them to acoustic steaming-induced drag forces and acoustic radiation force. Using a high-speed camera and particle-tracking technique, we observed that the drag force generated by the SAWs fulfilled the denudation mechanism. Additionally, due to the small attenuation coefficient in water, thermal absorption heating remains minuscule, preventing any thermal-induced damage.

Our device significantly reduced the time and labor of the denudation process. It also yielded proper denudation quality without oocyte loss. To ensure that SAWs do not damage oocytes, 40 oocytes denuded by 80 MHz SAWs, 25 oocytes denuded by 200 MHz SAWs, and 30 oocytes denuded by MP were inseminated by piezo-actuated ICSI. The 80-MHz, 200-MHz, and MP groups yielded comparable post-ICSI survival (82.5% vs. 84.0% vs. 83.3%, respectively), fertilization (80.0% vs. 80.0% vs. 83.3%,respectively), and blastulation rates (72.5% vs. 82.0% vs. 66.7%, respectively). Embryo morphokinetics were also not impacted. After transferring all blastocysts into recipient mice, 8 live births were achieved from the 80-MHz group, while 5 were achieved from the 200-MHz group.

Limitations, reasons for caution: Although PDMS is a popular material due to its high optical transparency and biocompatibility, adverse effects due to gas permeability and small-molecule adsorption cannot be excluded. Large-scale mouse embryo assays should be performed to assess the teratogenicity of PDMS. Operation parameters must be optimized for human COCs in clinical application.

Wider implications of the findings: Adopting widely used ultrasound techniques with emerging SAW technology is a major step toward advancing and standardizing oocyte denudation—a laborious yet delicate procedure. We predict it will be further integrated with AI and miniaturized robotics, modules specialized in gamete assessment, ICSI, and embryo evaluation in the near future.

Trial registration number: 'not applicable'

O-125 Development of an artificial intelligence embryo witnessing system to accurately track and identify patient specific embryos in a human IVF laboratory.

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Study question: Can convolutional neural networks (CNN) be used as a witnessing system to accurately track and identify patient specific embryos at the cleavage stage of development?

Summary answer: We developed the first artificial intelligence driven witnessing system to accurately track cleavage and blastocyst stage embryos in a human ART laboratory.

What is known already: There are reports of human errors in embryo tracking that have led to the births of children with different genetic makeup than their birth parents. Clinical practices rely on manual identification, barcodes or radio-frequency identification technology to track embryos. These systems are designed to track culture dishes but are unable to monitor developing embryos within the dish to help ensure an error-free patient match. Previously, we developed an AI witnessing system to track blastocysts with 100% accuracy. The goal of this study was to determine whether an AI witnessing system could be developed that accurately tracks cleavage stage embryos.

Study design, size, duration: A pre-developed deep neural network technology was first trained and tested on 4944 embryos images. The algorithm processed embryo images for each patient and produced a unique key that was associated with the patient ID at 60 hpi, which formed our library. When the algorithm evaluated embryos at 64 hpi it generated another key that was matched with the patient's unique key available in the library.

Participants/materials, setting, methods: A total of 3068 embryos from 412 patients were examined by the CNN at both 60 hpi and 64 hpi. These timepoints were chosen as they reflect the time our laboratory evaluates Day 3 embryos (60 hpi) and the time we move them to another dish and prepare them for transfer (64 hpi). The patient cohorts ranged from 3-12 embryos per patient.

Main results and the role of chance: The accuracy of the CNN in correctly matching the patient identification with the patient embryo cohort was 100% (CI: 99.1% to 100.0%, n = 412).

Limitations, reasons for caution: Limitations of this study include that all embryos were imaged under identical conditions and within the same EmbryoScope. Additionally, this study only examined fresh Day 3 embryos cultured over a span of 4 hours. Future studies should include images of fresh and frozen/thawed embryos captured using different imaging systems.

Wider implications of the findings: This study describes the first artificial intelligence-based approach for cleavage stage embryo tracking and patient specimen identification in the IVF laboratory. This technology offers a robust witnessing step based on unique morphological features that are specific to each individual embryo.

Trial registration number: This work was partially supported by the Brigham Precision Medicine Developmental Award (Brigham Precision Medicine Program, Brigham and Women's Hospital), Partners Innovation Discovery Grant (Partners Healthcare), and R01AI118502, and R01AI138800.

SELECTED ORAL COMMUNICATIONS

SESSION 37: THE ENDOMETRIUM IN IMPLANTATION EARLY PREGNANCY

29 June 2021 Stream 2 15:15 - 16:30

O-126 Endometrial microbiota composition is associated with reproductive outcome in infertile patients

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Study question: Is there an association between the composition of the endometrial microbiota and the reproductive outcomes in infertile patients undergoing in vitro fertilization (IVF)?

Summary answer: The composition of the endometrial microbiota (EM) prior to embryo transfer is associated with the different reproductive outcomes: live birth, no pregnancy or clinical miscarriage.

What is known already: The investigation of bacterial communities in the female reproductive tract using molecular methods has revealed the existence of a continuum microbiota that extends from the vagina to the upper genital tract. Previous evidence suggests the existence of an association between the vaginal and endometrial microbiome composition with reproductive and obstetrical outcomes. Specifically, the presence of specific pathogens together with low abundance of Lactobacilli has been associated with poor IVF outcomes.

Study design, size, duration: Multicentre prospective observational clinical study analysing the EM of infertile patients undergoing IVF (with maternal age ≤40) or ovum donation (≤50 years). A total of 452 infertile patients undergoing IVF/ovum donation were assessed for eligibility in 13 reproductive clinics in Europe, America, and Asia. The duration of the study was 30 months and the recruitment period extended between August 2017 and February 2019 (ct.gov 03330444).

Participants/materials, setting, methods: Endometrial fluid and endometrial biopsy were collected during a hormonal replacement therapy cycle after 5 days of progesterone (P) administration prior to a frozen embryo transfer cycle. Endometrial microbiota (EM) composition was analyzed using 16S rRNA gene sequencing using compositional data to transform scale-invariant values in both sample types. The EM in fluid and biopsy was associated with live birth, biochemical pregnancy, clinical miscarriage, or no pregnancy.

Main results and the role of chance: Of the 452 patients assessed, 44 did not meet the selection criteria and were excluded for the study and 66 patients

were lost to follow-up. Of the 342 remaining patients, 198 (57.9%) became pregnant [141 (41.2%) had a live birth, 27 (7.9%) had a biochemical pregnancy, 2 (0.6%) had an ectopic pregnancy, and 28 (8.2%) a clinical miscarriage], while 144 (42.1%) did not become pregnant. The baseline characteristics, clinical and embryological variables were homogeneous and no bias toward the clinical outcome categories was observed.

Our association study showed that the composition of the EM was associated with the reproductive outcome in both endometrial fluid and biopsy. A dysbiotic endometrial microbiota profile composed of *Atopobium*, *Bifidobacterium*, *Chryseobacterium*, *Gardnerella, Haemophilus, Klebsiella, Neisseria, Staphylococcus* and *Streptococcus* was significantly associated with unsuccessful outcomes, especially no pregnancy and clinical miscarriage. In contrast, *Lactobacillus* was consistently enriched in patients with live birth outcomes. The EM in endometrial fluid did not fully reflect that in endometrial biopsy, although their association with clinical outcome was consistent.

Limitations, reasons for caution: The main limitation was the small number of biochemical pregnancy and clinical miscarriage analysed. During transcervical collection of endometrial samples caution was taken to avoid contamination with the cervix although cervical contamination cannot be fully discarded.

Wider implications of the findings: Our data indicate that EM dysbiosis is associated with poor clinical outcome in ART. Thus, the EM composition before embryo transfer could be a useful biomarker to consider offering an opportunity to further improve diagnosis and treatment strategies.

Trial registration number: Clinical trials.gov 03330444

O-127 The endometrial tissue microbiota of women who did or did not achieve a live birth within 12 months after a first failed IVF/ICSI cycle

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Study question: After one failed IVF/ICSI cycle, does the endometrial microbiota composition differ between women who will or will not reach a live birth within 12 months?

Summary answer: The endometrial microbiota composition did not significantly differ in women with one failed IVF/ICSI cycle with or without live birth, but statistical power was low.

What is known already: Evidence for the presence of an indigenous endometrial microbiome is mounting, and its composition may be associated with implantation success. However, a 'core' endometrial microbiome has not yet been defined, and its role in embryo implantation is still poorly understood. Further investigation of this topic may allow improvement and personalisation of clinical care for infertile couples. Endometrial microbiome analysis in infertile women has not yet been performed using transcervically obtained endometrial tissue. Using endometrial tissue instead of swabs or fluid may increase the bacterial DNA yield and therefore the precision of microbiome analyses.

Study design, size, duration: Endometrial tissue was obtained from a cohort of 141 infertile women undergoing endometrial scratching within a randomised controlled trial (RCT) (SCRaTCH trial, NL5193/NTR5342). Briefly, women aged 18-44 years with failed implantation after one full IVF/ICSI cycle and planning a subsequent IVF/ICSI cycle, were eligible. Participants were followed-up until 12 months after randomisation, with the primary outcome being live birth, defined as the delivery of at least one live foetus after 24 weeks of gestation.

Participants/materials, setting, methods: Endometrial tissue was obtained with an endometrial biopsy catheter in the midluteal phase of a natural cycle preceding subsequent IVF/ICSI, snap-frozen and stored at -80°C until use. Total DNA was isolated from these biopsies, followed by 16S rRNA sequencing (V3-V4 region) to determine the endometrial microbiota composition. Positive (mock communities) and negative controls (DNA extraction and PCRs) were included. QIIME2 and DADA2 were used for the data analysis, followed by statistical analysis in R studio.

Main results and the role of chance: During the 12-month follow-up, 61/141 women (43.3%) reached a live birth. While endometrial microbiota profiles of all 141 women were analysed, only samples with ≥100 reads were included in the analysis, resulting in a total of 46 samples (32.6%) that were included in the analysis, which consisted of samples from 25 women who did not have and 21 women who did have a live birth within 12 months. The median number of reads per sample was not significantly different between the two groups (respectively 2,317 (IQR 651-19,031) and 1,335 (IQR 296-3,180), p=0.29 by Mann-Whitney test). The endometrial microbiota detected, were bacterial genera frequently reported within the vaginal microbiota (e.g. *Lactobacillus*, *Atopobium* and *Gardnerella*). A clear dominance of *Lactobacillus* (relative abundance 55-100%, n=22) or an unclassified bacterium genus (relative abundance 52-76%, n=18) was observed in the majority of the samples; however, this dominance was not associated with the outcome of live birth. In addition, the samples dominated by Lactobacillus genera were mostly dominated by one species of *Lactobacillus* each (*L. crispatus*, *L. iners*, *L. gasseri* or *L. jensenii*).

Limitations, reasons for caution: The low biomass and the low ratio of bacterial versus human DNA in endometrial tissue were limiting factors in endometrial microbiota analysis. Furthermore, tissue was obtained transcervically, and contamination with vaginal/cervical microbiota could therefore have occurred. In the SCRaTCH trial no vaginal swabs were taken to serve as internal controls.

Wider implications of the findings: Future endometrial microbiota studies should consider the use of samples with a lower proportion of human DNA to maximize bacterial DNA yield. Furthermore, for endometrial microbiota research, sampling devices avoiding cervicovaginal contamination are desirable and may be developed in the future.

Trial registration number: SCRaTCH trial, NL5193/NTR5342

O-128 Intra-cycle alterations of the uterine microbiota in patients with recurrent miscarriage or recurrent implantation failure and healthy controls

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Study question: Uterine microbiota: are there differences within three major time points of the menstrual cycle in healthy controls, recurrent miscarriage (RM) and recurrent implantation failure (RIF) patients?

Summary answer: Compared to controls, RM and RIF patients showed an altered uterine microbiota throughout the menstrual cycle, with a lower dominance of lactobacilli.

What is known already: In contrast to the former notion of a sterile womb, bacterial colonization in the uterus and the placenta has been demonstrated. Studies showed that Lactobacillus-dominated endometrial microbiota correlate with reproductive success. Moreover, the presence of non-Lactobacillus-dominated microbiota, especially with detection of Gardnerella and Streptococcus in the endometrial fluid, seems to be associated with lower implantation-, ongoing pregnancy- and live birth-rates. However, intra-cycle variations in healthy women as well as possible alterations in patients with RM or RIF remain unknown.

Study design, size, duration: In total, n=20 RM patients (≥ 3 consecutive miscarriages), n=20 RIF patients (≥3 fresh or frozen embryo transfers with negative serum hCG, good quality embryos) and n=10 healthy controls (no pregnancy) were included in this study. All patients had a 28 day menstrual cycle. During follicular, ovulatory and luteal-phase, after a thorough cleaning of the cervix, a flexible catheter was introduced into the uterine cavity and a uterine flushing with 1ml of NaCl was performed.

Participants/materials, setting, methods: Bacterial DNA was extracted using a QIAamp DNA kit (Qiagen) in combination with a PrecellysR24 homogenizer (Peqlab, Erlangen, Germany) according to the manufacturer's instructions. The V3-V4 region of the bacterial 16S rRNA gene was amplified. Samples were

pooled in equimolar ratios and progressed to pyrosequencing using an Illumina MiSeq se-quencer with MiSeq Kit V2 (250 bp paired-end). Analysis of 16S rRNA data, including alpha- and beta-diversity, were calculated using the phyloseq package in R.

Main results and the role of chance: For the Shannon index (species richness and evenness) a significant decrease during the ovulation period was shown in the control group, indicating a more uniform microbiota ($p < 0.05$). This loss of diversity was not shown in RIF and RM patients. Overall, we could observe a higher similarity in taxonomic distribution in RM compared to the RIF patients. Longitudinal dynamics included increases in Firmicutes (CTRL and RM only) and a concomitant loss of Proteobacteria. Notably, significant amounts of bacteroides were only detected in the RIF patients. Actinobacteria were more frequent in both, RM and RIF as compared to controls.

Limitations, reasons for caution: To minimize the impact of a potential contamination, we performed pre-experiments with paired samples both from the vaginal fornix and the endometrial cavum and could show a significant difference in overall microbiome configuration. However, the route of sample can still be prone to contamination.

Wider implications of the findings: For the first time, we were able to show cycle-dependent alterations in the endometrial microbiome. These findings underline the role of an altered endometrial microbiome as a cause for RM and RIF and can contribute to the future establishment of therapeutic strategies in cases of a dysbalanced microbiome.

Trial registration number: Drks00020803

O-129 Lactobacillus deplete vaginal microbial composition is associated with chromosomally normal miscarriage and local inflammation.

K. Grewal[1], Y. Lee[1], A. Smith[2], J. Brosens[3], M. Al-Memar[1], T. Bourne[4], S. Kundu[1], D. MacInytre[4], P. Bennett[4]

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Study question: To investigate the vaginal microbial composition and the local immune response in chromosomally normal and abnormal miscarriages and compare this to uncomplicated pregnancies delivering at term.

Summary answer: We show that euploid miscarriage is associated with a significantly higher prevalence of *Lactobacillus* spp. deplete vaginal microbial communities compared to aneuploid miscarriage.

What is known already: Emerging evidence supports the role of the vaginal microbiota in adverse pregnancy outcome, but the underlying mechanisms are poorly understood. A dominance of *Lactobacillus* spp. in pregnancy provides protection against pathogenic bacteria by producing lactic acid and antimicrobial compounds. A depletion in Lactobacillus spp. is often linked to adverse pregnancy outcomes.Current work also implicates the reproductive tract microbiota as a key modulator of local inflammatory and immune pathways. We have previously shown that miscarriage is associated with vaginal dysbiosis but without knowledge of the cytogenetic status of those miscarriages or the local immune profile.

Study design, size, duration: This study was a prospective observational cohort study based at Queen Charlotte's & Chelsea Hospital, Early Pregnancy Unit, London between March 2014-February 2019. Vaginal swabs were collected from the posterior vaginal fornix of 167 patients.

Participants/materials, setting, methods: We used 16S rRNA gene based metataxonomics to interrogate the vaginal microbiota in a cohort of 167 women, 93 miscarriage patients (54 euploid and 39 aneuploid using molecular cytogenetics) and 74 women who delivered at term and correlate this with the aneuploidy status of the miscarriages. We also measured the concentrations of IL-2, IL-4, IL-6, IL-8, TNF-α, IFN-γ, IL-1β, IL-18 and IL-10 in cervical vaginal fluid using Human Magnetic Luminex Screening Assay (8-plex).

Main results and the role of chance: We show that euploid miscarriage is associated with a significantly higher prevalence of *Lactobacillus* spp. deplete vaginal microbial communities compared to aneuploid miscarriage (P=0.008). In women having *Lactobacillus* spp. deplete vaginal microbial communities, euploid miscarriage associates with higher concentrations of pro-inflammatory cytokines IL-1β, IL-8, IL-6 (P<0.001, P=0.01 and P<0.001 respectively) and lower concentrations of anti-inflammatory cytokines IL10 (P<0.001) when compared to viable term pregnancy. We identified *Prevotella bivia* and *Streptococcus* as particularly common in euploid miscarriage and as drivers of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α). Co-occurrence network analyses revealed low levels of co-occurrence between *Lactobacillus crispatus* and other organisms and strong co-occurrence between Streptococcal species. Our data show a combination of both an adverse vaginal microbiota and a cytokine response to it influences early pregnancy outcome. Although this may be a reflection of intrinsic maternal immune response, it appears that the cytokine response is largely driven by the bacterial taxa present in the vagina, which presents an opportunity for specific, directed intervention. The negative co-occurrence between *L.crispatus* and all other organisms suggests a possible therapeutic role for probiotics containing this organism. The influence of Streptococci also suggests a potential benefit of targeted antibiotics with probiotics for some patients.

Limitations, reasons for caution: There were no longitudinal samples in this cohort and our results are based on the assumption that the vaginal microbial composition is stable throughout the first trimester.Future longitudinal studies with larger sample sizes are needed to corroborate these findings and provide insights to the mechanisms that trigger the inflammatory response.

Wider implications of the findings: These findings support the hypothesis that the vaginal microbiota plays an important aetiological role in euploid miscarriage and may represent a target to modify the risk of pregnancy loss.

Trial registration number: n/a

O-130 The influence of vaginal microbiota on frozen blastocyst implantation after transfer: a prospective study through next-generation 16S rRNA sequencing

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Study question: A prospective study to investigate the relationship between the composition of vaginal microbiota through next-generation sequencing and the efficacy of single frozen blastocyst transfer in the same cycle.

Summary answer: Dominant presence of lactobacillus and other lactate producing microbes in the upper vagina was highly correlated with implantation of transferred blastocyst in this pilot study.

What is known already: Next-generation sequencing of 16S rRNA detected microbes in the uterine cavity and recent studies indicated that dominant presence of *Lactobacillus* correlated highly with successful implantation of the transferred embryos. Aberrant vaginal microbiota has been known to cause poor obstetrical outcomes, however little is known for its effect on embryo implantation in assisted reproduction.

Study design, size, duration: A prospective study with 25 female subjects transferring a frozen blastocyst using autologous oocyte, over 14 months period in 2019-2020.

Participants/materials, setting, methods: 25 female patients without tubal and uterine pathology and no history of multiple miscarriages and implantation failures were recruited with consent at a private ART clinic. Transdermal estrogen was used to prepare endometrium. Upper vaginal fluid was obtained in follicular phase of the the same cycle and analyzed through next-generation sequencing, but the result was reported after pregnancy confirmation. Single frozen blastocyst transfer and standard luteal phase support were performed. Institutional IRB approved the protocol.

Main results and the role of chance: The mean age was 36.2 y.o.(range 29-41 y.o.), and 14 gestational sacs (implantation rate 56%), and 3 miscarriage (21.4%) were observed. Next-generation sequencing for 16S rRNA revealed average 69.2% presence of *Lactobacillus* (0-100%) and average 78.0% (0.2-100%) lactate producing microbes (LPM: *Lactobacillus, Bifidobacterium, Streptococcus,* and *Enterococcus*) in the vaginal fluid. Using 90% as cut-off, implantation rates were 11/15 (73.3%) and 3/10 (30%) with *Lactobacillus* dominant and non-dominant, 12/16 (75%) and 2/9 (22.2%) with LPM dominant and non-dominant microbiota, respectively. The difference in each group were not statistically significant. The relative risks for pregnancy were 2.63 (95%CI 1.03-6.67, P=0.04)

for *Lactobacillus* and 3.11 (95%CI 1.24-7.79, *P*=0.02) for LPM. As for ROC analysis for embryo implantation and dominant microbes, AUC and associated criterion were 0.62 and 90.7% (sensitivity 78.6%, specificity 72.7%) for *Lactobacillus,* 0.69 and 96.6% (sensitivity 85.7%, specificity 72.7%) for LPM, respectively. The difference of AUC was not significant (*P*=0.24).

Limitations, reasons for caution: Despite prospective nature of the study, small sample size limited the analytical power of the study. Aneuploidy screening was not performed to remove confounding factor.

Wider implications of the findings: Our pilot study revealed possible relationship between vaginal microbiota and embryo implantation. Dominance of *Lactobacillus* or other lactate producing microbes may be advantageous for successful ART. Sampling vaginal fluid for microbe analysis is less invasive than endometrial sampling and can obtain more abundant RNA with possible higher accuracy of analysis.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 38: UTERINE DISORDERS: MEDICAL APPROACHES

29 June 2021 Stream 3 15:15 - 16:45

O-131 The effect of time since surgical diagnosis of endometriosis on treatment outcomes with relugolix combination therapy in women with endometriosis-associated pain: SPIRIT program

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Study question: To assess the efficacy of Relugolix-CT vs placebo in women who were surgically diagnosed with endometriosis <5 and >= 5 years ago.

Summary answer: Treatment outcomes did not differ for dysmenorrhea and daily functioning between subgroups of patients with <5 years or ≥5 years since surgical diagnosis.

What is known already: Time since clinical manifestation and diagnosis of endometriosis may influence the treatment success of patients with endometriosis-associated pain. SPIRIT 1 and 2 were randomized, double-blind, placebo-controlled Phase 3 studies of Relugolix-CT (relugolix 40 mg, estradiol 1 mg, norethindrone acetate 0.5 mg) in premenopausal women (age 18–50 years) with surgically diagnosed endometriosis and a history of moderate-to-severe dysmenorrhea and non-menstrual pelvic pain (NMPP). These studies previously demonstrated that Relugolix-CT significantly reduced dysmenorrhea and NMPP, and improved daily functioning measured by the Endometriosis Health Profile-30 (EHP-30) pain domain score vs placebo over 24 weeks.

Study design, size, duration: Premenopausal women with surgically diagnosed endometriosis and moderate-to-severe dysmenorrhea and NMPP at baseline were randomized 1:1:1 to 24 weeks of treatment with once daily oral Relugolix-CT, delayed Relugolix-CT (relugolix 40 mg monotherapy for 12 weeks followed by Relugolix-CT for 12 weeks), or placebo. The proportion of dysmenorrhea and NMPP responders at Week 24/End-of-Treatment (EoT), based on daily Numerical Rating Scale (NRS), and analgesic use status were co-primary endpoints.

Participants/materials, setting, methods: Pooled SPIRIT 1 and 2 data of patients who received 24 weeks of treatment with once daily Relugolix-CT (N=418) or placebo (N=416) are presented. Outcomes for the delayed Relugolix-CT group were only for the safety assessment and therefore not reported here. Analyses of NRS scores for dysmenorrhea, NMPP, and EHP-30 pain domain score were carried out in the subgroups of patients with time since diagnosis of <5 years (N=579) and ≥5 years (N=255).

Main results and the role of chance: Baseline demographics and clinical characteristics were comparable between the time since diagnosis subgroups except for a numerically higher mean age in the ≥5-years subgroup. Mean time since diagnosis (standard deviation) was 2.1 (1.5) years with both Relugolix-CT and placebo for <5-years subgroup, and 8.0 (2.8) and 7.8 (2.3) years, respectively, for ≥5-years subgroup.

In Relugolix-CT-treated patients, mean NRS score for dysmenorrhea decreased from 7.5 (severe) to 1.8 (mild) in the <5-years subgroup and from 6.9 (moderate) to 1.8 (mild) in the ≥5-years subgroup with a significant difference to placebo (p<0.0001, both subgroups), and demonstrating 74.8% and 72.7% reduction in pain from baseline to Week 24/EoT, respectively. Mean NRS score for NMPP decreased from 6.0 (moderate) to 3.0 (mild) with a significant difference compared with placebo (p<0.0001), equating to 48.8% pain reduction in the <5-years subgroup, and from 5.6 (moderate) to 2.7 (mild) equating to 51.5% pain reduction (p=0.089) in the ≥5-years subgroup. Improvement of daily functioning as measured by EHP-30 pain domain score was significantly greater with Relugolix-CT vs placebo in both subgroups, with decrease in EHP-30 pain score from 59.1 to 24.0 in the <5-years subgroup, and from 57.4 to 21.1 in the ≥5-years subgroup (p<0.0001, both subgroups).

Limitations, reasons for caution: A lower number of patients were included into the subgroup with ≥5-years since surgically diagnosed endometriosis. Five-year dichotomy was close to the mean time since surgical diagnosis in the studies and to certain extent is arbitrary. Furthermore, time since surgical diagnosis is not the same as time since symptom onset.

Wider implications of the findings: In women with endometriosis-associated pain, Relugolix-CT vs placebo significantly reduced dysmenorrhea and improved daily functioning in both groups: with surgical diagnosis of <5 years or ≥5 years. Substantial decrease in NMPP was also observed and was significantly different to placebo in the <5-years subgroup.

Trial registration number: NCT03204318 and NCT03204331

O-132 Sustained efficacy and safety of relugolix combination therapy in women with endometriosis-associated pain: SPIRIT 52-week data

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Study question: To assess the long-term (52-week) efficacy and safety of relugolix combination therapy (Relugolix-CT) in the treatment of endometriosis-associated pain.

Summary answer: Relugolix-CT demonstrated a sustained improvement of endometriosis-associated pain and maintenance of bone mineral density (BMD) over the extension treatment period. It was well tolerated.

What is known already: Endometriosis is a chronic condition characterized by symptoms of menstrual and non-menstrual pain, and dyspareunia, which have a substantial impact on women's lives. SPIRIT 1 and 2 were Phase 3, randomized, double-blind, placebo-controlled studies of once-daily Relugolix-CT (relugolix 40 mg, estradiol 1 mg, norethindrone acetate 0.5 mg) in premenopausal women (age 18–50 years) with surgically diagnosed endometriosis and moderate-to-severe dysmenorrhea and non-menstrual pelvic pain (NMPP) at baseline. These trials demonstrated a significant improvement of dysmenorrhea, NMPP and dyspareunia in women treated with Relugolix-CT, with a minimal decline in BMD vs placebo over 24 weeks.

Study design, size, duration: Women who completed the 24-week pivotal studies (SPIRIT 1 and 2 trials) were eligible to enroll in an open-label, single-arm, long-term safety and efficacy extension study for an additional 80 weeks. All women received once-daily oral Relugolix-CT. Analyses were done based on original randomization in pivotal studies: Relugolix-CT, delayed Relugolix-CT (relugolix 40 mg alone for 12 weeks, then Relugolix-CT for 12 weeks), or placebo. Here, 52-week efficacy and safety outcomes are presented.
Participants/materials, setting, methods: The primary endpoints were the proportion of dysmenorrhea and NMPP responders at Week 52, based on daily Numerical Rating Scale (NRS) scores (0=no pain, 10=worst pain imaginable). A responder was a woman who achieved a predefined, clinically meaningful reduction from baseline in NRS score with no increase in analgesic use. Secondary efficacy endpoints included change in Endometriosis Health Profile-30 (EHP-30) pain domain scores, and analgesic/opioid use. Safety endpoints included adverse events (AEs) and BMD evaluation.
Main results and the role of chance: Of 1261 randomized patients, 1044 completed the primary studies; 802 enrolled in the long-term extension and 681 completed 52 weeks of treatment. Baseline demographics and clinical characteristics of the extension population were consistent with those of the original randomized population.

Sustained improvement of endometriosis-associated pain was demonstrated with Relugolix-CT through 52 weeks, the proportion of responders for dysmenorrhea was 84.8% and 73.3% for NMPP.

NRS least squares (LS) mean scores for dysmenorrhea and NMPP decreased from 7.4 (severe) and 6.0 (moderate) at SPIRIT study baseline to 1.3 (mild) and 2.2 (mild) at Week 52, equating to 82.8% and 62.9% reduction in dysmenorrhea and NMPP, respectively. Mean NRS for dyspareunia decreased from 5.9 (moderate) to 2.4 (mild), demonstrating 60.1% reduction with Relugolix-CT.

Daily functioning measured by the EHP-30 pain domain score was improved (–38.1 point) and the majority of women (85.6%) were opioid-free at Week 52. There was no disproportionate increase in the incidence of AEs in the Relugolix-CT group with no new safety signals identified through the 52 weeks. BMD was preserved over the extension period with overall LS mean change from baseline to Week 52 of –0.83% (95% CI: –1.34, –0.32) for lumbar spine in the Relugolix-CT group.
Limitations, reasons for caution: The study was conducted as an open-label study without a control group over the 28 weeks of the extension period.
Wider implications of the findings: Relugolix-CT demonstrated a sustained improvement of dysmenorrhea, NMPP, and dyspareunia, and reduced pain-related functional limitations and the need for opioids over 52 weeks in women with moderate-to-severe endometriosis-associated pain. Relugolix-CT was generally well tolerated and associated with minimal BMD loss after treatment initiation followed by BMD maintenance over 52 weeks.
Trial registration number: NCT03654274

O-133 Effect of non-surgical treatments on the size of endometrioma: A systematic review and Meta-Analysis

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Study question: This review aims to determine the effectiveness of medical preparations in the management of endometrioma size
Summary answer: Medical treatment from different groups significantly reduces the endometrioma size, and relives painful symptoms
What is known already: Endometrioma is usually found in more severe stage of endometriosis and likely to be treated surgically. However, surgery of an endometrioma always destroys ovarian tissue and eventually reduces the ovarian reserve.
Study design, size, duration: Systematic review and analysis.The studies included 810 women in the intervention groups and 442 in the control groups
Participants/materials, setting, methods: 810 women included in the intervention groups . 442 in the control . All were women with endometrioma; 30-60% had pelvic pain. age was 33.4 years (range 27-39.8).

Four electronic databases search PubMed, Embase, CINAHL Google Scholar from each database inception date until 10 .9. 2019 using the specific search term. randomized and nonrandomized were included. trials comparing different medications for endometiotic cyst medical treatment, without language restriction and in consultation with a search methodologist
Main results and the role of chance: We found 3121studies from the initial search and out of which 3102 were excluded due to they did not fulfil the selection criteria, We included 14 studies for the systematic review leaving 4/14 for the meta-analysis. 14 final studies included for the systematic review out of which 6/14 were uncontrolled. The majority of the studies were prospective (10/14), and four were RCTs. 2/4 (n=395). controlled studies patient treated with a low-dose (OCP) or NET and dienogest +EE compared with patients treated with placebo, the endometrioma size significantly reduced (odds ratio[OR]1.32;95%CI [0.61,2.84],2studies, 255women, I= 0%).2/4 studies included in meta-analysis patients treated with dienogest versus norethendrone or ethinyl estradiol [EE] plus dienogest (SMD - 6.73;95%CI[-16.53, 3.06],2studies,185 women, I= 83%)
Conclusions: Medical treatment from different groups significantly reduces the endometrioma size, and relives painful symptoms.
Limitations, reasons for caution: Despite the nature of the randomized includedstudies, the results of this study are still subjected to confounders relating to clinical and statistical heterogeneity. the studies reported the outcomes differently
Wider implications of the findings: medical treatment can be given the surgery can be avoided, therefore preserve the ovarian tissue. There is limited controlled study to examine the effectiveness of the treatment for endometrioma.
Trial registration number: ----------

O-134 Cochrane review on the effect of pentoxifylline for endometriosis

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Study question: To assess the effect of pentoxifylline, a methyl-xanthine with anti-inflammatory effects, for the management of premenopausal women with endometriosis.
Summary answer: There is not enough evidence to support the use of pentoxifylline in the management of premenopausal women with endometriosis to improve fertility and pain outcomes.
What is known already: Endometriosis is a chronic, inflammatory condition that occurs mainly during the reproductive years. It is characterized by endometrium-like tissue developing outside the uterine cavity. This endometriotic tissue development is dependent on estrogen produced primarily by the ovaries and partially by the endometriotic tissue itself and, therefore, hormonal management is traditionally used. In light of the body of evidence suggesting an immunological component to the pathophysiology of endometriosis, the anti-inflammatory agent pentoxifylline has been proposed as an alternative therapeutic agent.
Study design, size, duration: A Cochrane systematic review and meta analysis was performed. Electronic searches of the Cochrane Gynaecology and Fertility Specialised Register of Controlled Searches, CENTRAL, MEDLINE, EMBASE, PsycINFO, CINAHL and AMED OVID were conducted to December 2020 to identify relevant randomised controlled trials (RCTs). In addition, electronic searches were conducted on the Epistemonikos database, Human Reproduction, Web of Knowedlge, OpenGrey, LILACS, Pubmed and Google.
Participants/materials, setting, methods: Participants: premenopausal women with endometriosis via laparoscopy/laparotomy. For extent of endometriosis, grades according to the AFS/rASRM scoring system were used.
Intervention: pentoxifylline treatment for any period of time
Comparisons: placebo, no treatment, medical treatment, surgical treatment.
Two independent authors screened studies and extracted data. Risk ratios were calculated for dichotomous data (Peto odds ratio for low event rates) and mean differences (MD) for continuous data, with 95% confidence intervals (CI).

Main results and the role of chance: Five RCTs were included, involving 415 participants.

Pentoxifylline vs placebo

No trials reported on live birth or reduction of pain. We are uncertain whether pentoxifylline affects the clinical pregnancy rate (Peto OR 1.53, 95% CI 0.89 to 2.63; 3 RCTs, n=285; $I^2 = 0\%$; very low-quality evidence), recurrence rate (Peto OR 0.83, 95% CI 0.26 to 2.60; 1 RCT, n=121; very low-quality evidence), or miscarriage rate (Peto OR 1.99, 95% CI 0.20 to 19.37; 2 RCTs, n=164; $I^2 = 0\%$; very low-quality evidence).

Pentoxifylline vs no treatment

We are uncertain whether pentoxifylline impacts on pain reduction when compared to no treatment at one month (MD -0.36, 95% CI -2.08 to 1.36; 1 RCT; n = 34; very low-quality evidence), two months (MD -1.25, 95% CI -2.67 to 0.17; 1 RCT; n = 34; very low-quality evidence) or three months (MD -1.60; 95% CI -3.32 to 0.12; n = 34; very low-quality evidence). No studies reported on live birth.

Pentoxifylline vs medical treatment

One study compared pentoxifylline with the combined contraceptive pill, but could not be included in the meta-analysis, as it was unclear if the data were presented as +/- standard deviation.

Pentoxifylline vs surgical treatment

No study reported on this comparison

Limitations, reasons for caution: Based on the GRADE criteria, the quality of evidence was classified as very low with issues arising due to risk of bias and imprecision. Four studies did not apply the intention-to-treat principle. None of the studies reported on live birth rate, one of the primary outcomes of the review.

Wider implications of the findings: Future research should prioritise live birth and overall pain as the primary outcome and include patients with all endometriosis severity types. All included studies compared pentoxifylline with placebo or no treatment after surgery, which highlights the need for more types of comparisons, such as to hormonal contraception.

Trial registration number: Not applicable

O-135 Long term secondary efficacy of linzagolix for heavy menstrual bleeding (HMB) due to uterine fibroids (UF): 52-week results from two placebo-controlled, randomized, phase 3 trials

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Study question: Are symptomatic improvements in women with UF observed after 24 weeks of linzagolix treatment with or without add-back therapy (ABT) maintained over 52 weeks?

Summary answer: Improvements in anemia, pain and quality of life previously reported at 24 weeks were maintained at 52 weeks.

What is known already: We previously reported that partial or full suppression of estradiol (E2) with once daily doses of either 100 or 200 mg linzagolix for 24 weeks, with or without ABT, were effective in reducing heavy menstrual bleeding associated with uterine fibroids, improving other symptoms such as pain and anemia and improving quality of life. Here we report the maintenance of effect on secondary endpoints after 52 weeks of treatment.

Study design, size, duration: Linzagolix is an investigational, oral GnRH antagonist being developed to treat HMB due to UF. PRIMROSE 1 (P1, USA, NCT03070899) and PRIMROSE 2 (P2, Europe and USA, NCT03070951) are randomized, double-blind, placebo-controlled Phase 3 trials, with essentially identical design, investigating the efficacy and safety of linzagolix with and without hormonal add-back therapy (ABT: 1 mg estradiol/0.5 mg norethindrone acetate) once daily for 52 weeks.

Participants/materials, setting, methods: Participants had HMB due to UF (>80mL menstrual blood loss (MBL)/cycle) and were equally randomized to: placebo, linzagolix 100mg, linzagolix 100mg+ABT, linzagolix 200mg, or linzagolix 200mg+ABT. After 24 weeks, subjects originally randomized to placebo or linzagolix 200mg were switched to linzagolix 200mg+ABT except in P1 where 50% placebo subjects continued placebo until 52 weeks. Secondary efficacy assessments included hemoglobin, pain (0–10 numeric rating scale) and health related quality of life (HRQL) on the UF-QoL questionnaire.

Main results and the role of chance: P1 trial subjects (n=526) had a mean age of 42 years, pain score of 6.6 and HRQL total score (0–100) of 36.4 and 63% were Black. P2 trial subjects (n=511) had a mean age of 43 years, pain score 4.8 and HRQL total score of 46.1 and 5% were Black. Mean baseline MBL was about 200 mL per cycle in both studies. In both trials, significant improvements compared to placebo observed at week 24 for secondary endpoints, including pain, anemia and QoL in all linzagolix treatment groups were maintained at 52 weeks.

Mean±SD hemoglobin levels in anemic patients (<12 g/dL) increased from baseline by 1.7±1.9, 1.9±1.7, 2.2±2.4, 2.7±1.9 in P1 and 1.2±1.9, 2.9±1.8, 2.4±2.1, 3.0±1.4 in P2 in the 100mg, 100mg+ABT, 200mg/200mg+ABT, 200mg+ABT groups, respectively, compared to 0.6±1.8 with placebo (P1).

Mean±SD change from baseline in pain scores were -3.3±3.1, -2.7±3.2, -2.6±3.0, -3.9±3.2 in P1 and -2.6±3.1, -2.6±2.8, -3.0±2.6, -2.8±3.0 in P2 in the 100mg, 100mg+ABT, 200mg/200mg+ABT, 200mg+ABT groups, respectively, compared to -0.4±2.5 with placebo (P1).

Mean±SD change in HRQL total scores were 25.0±26.2, 34.2±30.1, 29.7±29.2, 38.3±29.2 in P1 and 16.8±24.0, 29.6±23.2, 31.9±26.8, 30.7±26.0 in P2 in the 100mg, 100mg+ABT, 200mg/200mg+ABT, 200mg+ABT groups, respectively, compared to 14.6±23.9 with placebo (P1).

Limitations, reasons for caution: Here we report data in both trials up to 52 weeks of treatment. No statistical comparisons were done at 52 weeks (the primary analysis was done after 24 weeks treatment). Post-treatment follow-up will provide more information in symptom recurrence after stopping treatment.

Wider implications of the findings: All linzagolix treatments provided sustained benefit. Two regimens previously identified for potential long-term treatment, 200mg with ABT and 100mg without ABT, provided sustained improvements of anemia, pain and associated quality of life. These different treatment regimens could be important to address the diverse needs of women suffering from uterine fibroids.

Trial registration number: ClinicalTrials.gov: NCT03070899, NCT03070951

O-136 Once-daily relugolix combination therapy results in sustained reduction in symptoms and improved quality of life in women with uterine fibroids treated over 52 weeks

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Study question: What is the effect of relugolix combination therapy (Relugolix-CT) on symptom burden and health-related quality-of-life (HR-QoL) in patients with uterine fibroids (UF) over 52 weeks?

Summary answer: Relugolix-CT demonstrated sustained, clinically meaningful improvement in patient-reported symptom severity and HR-QoL over 52 weeks in women with UF.

What is known already: In LIBERTY 1 and 2 randomized clinical trials, once-daily Relugolix-CT (40 mg relugolix, an oral gonadotropin-releasing hormone

receptor antagonist, estradiol 1 mg, norethindrone acetate 0.5 mg) significantly reduced menstrual blood loss (MBL) and UF-associated pain versus placebo in women with UF and heavy menstrual bleeding (HMB), and was well tolerated, with bone mineral density (BMD) preservation through 24 weeks. In the LIBERTY long-term extension study, a sustained reduction in MBL was observed along with no new safety signals and BMD maintenance through 52 weeks. Relugolix-CT was previously shown to significantly improve patient-reported symptom severity and HR-QoL through 24 weeks.

Study design, size, duration: In the LIBERTY 1 and 2 studies, 770 premenopausal women with ultrasound-documented clinically significant UF and alkaline-hematin documented HMB were randomized 1:1:1 to Relugolix-CT for 24 weeks, relugolix 40 mg for 12 weeks followed by Relugolix-CT for 12 weeks, or placebo for 24 weeks. Women who completed the pivotal studies were eligible to enroll in the 28-week extension study (N=477 enrolled). Patients included in the extension study received open-label once-daily Relugolix-CT.

Participants/materials, setting, methods: Changes from baseline to Weeks 24 and 52 in the Uterine Fibroid Symptom (UFS)-QoL symptom severity scale, Bleeding and Pelvic Discomfort scale (BPD; assessing distress due to HMB, passing blood clots, pelvic pressure/tightness); and HR-QoL (sub)scales were assessed. Higher symptom severity and BPD scores reflect higher severity and distress, respectively; higher HR-QoL scores indicate better outcomes. Least-squares mean changes were provided for the original pivotal-study randomized treatment groups Relugolix-CT and placebo.

Main results and the role of chance: A sustained improvement in symptom severity was observed for the Relugolix-CT group from baseline to Weeks 24 and 52, with LS mean changes of –36.9 and –37.3 points, respectively. In patients initially treated with placebo, small changes were observed at Week 24 (–10.8 points), whereas a greater reduction from baseline was demonstrated at Week 52 (–35.0 points) after transitioning to Relugolix-CT.

Considering the BPD, measuring distress from key UF symptoms, LS mean changes of –50.9 and –51.3 points were observed in the Relugolix-CT group from baseline to Week 24 and 52, respectively. In patients initially treated with placebo, small changes were observed at Week 24 (–15.9 points), whereas a greater reduction from baseline was demonstrated at Week 52 (–48.6 points) after transitioning to Relugolix-CT.

Treatment with Relugolix-CT also resulted in a sustained improvement in different aspects of HR-QoL. LS mean total HR-QoL score increased from baseline to Weeks 24 and 52 by 40.8 and 40.4 points, respectively. In patients initially treated with placebo, small changes were observed at Week 24 (11.4 points), whereas a greater increase from baseline was demonstrated at Week 52 (39.0 points) after transitioning to Relugolix-CT at Week 24.

Limitations, reasons for caution: The 28-week LIBERTY long-term extension study was non-comparative. Analyses were performed in a subset of patients who completed the LIBERTY pivotal studies and were eligible for the extension study. However, it is important to note that the demographic and baseline disease characteristics were similar between pivotal and long-term extension populations.

Wider implications of the findings: Relugolix-CT resulted in a sustained reduction in UF symptom burden, particularly in terms of distress from key UF symptoms, and a sustained improvement of HR-QoL over 52 weeks. Improvements observed after transitioning from placebo to Relugolix-CT at 24 weeks confirmed the positive effect of Relugolix-CT on UF-associated symptoms and HR-QoL.

Trial registration number: NCT03412890

INVITED SESSION

SESSION 39: IVF ADD-ONS: IS THE JURY STILL OUT? A DEBATE

29 June 2021 Stream 1 17:00 - 18:00

O-047 For: Novel treatments can be used to increase efficiency and personalisation, if validated by big data

M. Meseguer Escriva[1]

[1]*Instituto Valenciano de Infertilidad, IVF Laboratory, Valencia, Spain*

O-048 Con: Add-ons should not be offered to patients without solid evidence from clinical trials

J. Harper[1]

[1]*Institute for Women's Health, Professor of Reproductive Science, London, United Kingdom*

INVITED SESSION

SESSION 40: MEDICINE AND MACHINE: WILL COMPUTERS TAKE OVER MAR?

29 June 2021 Stream 2 17:00 - 18:00

O-049 How is AI changing the way we make babies?

N. Zaninovic[1]

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Abstract text

ART is one of the fastest-growing fields in medicine. Besides the advancements in the hormonal stimulation and treatment regimes, the laboratory aspect of IVF showed emerged development in science and especially technology. The emphasis of future IVF treatments and laboratory procedures is on the non-invasive methods of embryo evaluation and selection. The application of Artificial Intelligence (AI) in daily life prompts us to evaluate this technology in this context. The AI is one of the main candidates to drive future IVF not only in the lab but also in all aspects of the IVF procedures. The benefit of AI technology involves "big data" analysis, objectivity, standardization, and precision. The most advancement of AI application is in the IVF lab where technology can be used as a tool to enhance our ability to select the "best" viable embryo objectively. As a new technology, it is important to evaluate it on the large scale. The AI technology will also enable us to standardize clinical parameters of patient reproductive potential and objectively predict each patient reproductive outcome per cycle. Ultimately, AI will help us to achieve the personalized and precision medicine needed in our field.

O-050 Virtual reality applications in ultrasound

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Abstract text

Surgical simulation has been introduced in surgical training since 1920 following the example of airline and military industry. Recently, the Association of American Medical Colleges commented on the increased use of simulation in different medical specialties, recognizing its potential ability to improve patients safety and to enhance healthcare in general. Historically, graduating obstetrics and gynecology residents have been expected to learn following the adage 'see one, do one, teach one', which required a high volume experience. Nowadays, as the complexity of procedures increases and surgical volume decreases, in order to acquire surgical skills rapidly there is the need to integrate surgical and ultrasound virtual simulation into modern residency training alongside traditional teaching methods. In addition, recent changes in the field (e.g. work-hours restrictions, decreased bedside teaching) have high lightened these necessities.

Ultrasonography is a skill that requires a complex interplay of motor skills and visual-cognitive skill. The learning process include two steps: an initial global assessment followed by a focal search for pathology and key landmarks. Recently more attention has been paid to design increasingly sophisticated simulators for ultrasound in obstetrics and gynecology. These are broadly divided into to major categories: physical mannequins and virtual reality simulators. The physical mannequins practically consist in low cost silicon dummies that enable learners to discover basic anatomical and pathological findings using real ultrasound equipment. This type of simulators allows the trainees to learn haw to use the

equipment in order to obtain proper scans. However, the case complexity is limited due to the low fidelity of the physical mannequins. In addition there is no automated feedback and a supervisor is always needed during the practice.

The virtual reality simulators usually rely on haptic devices or physical mannequins that record probe movements during transabdominal or transvaginal scans and combined them with computer-animated/real ultrasound images. Instructions and automated feedback are provided during the practice. They often contains multiple cases allowing the trainees to be exposed to different clinical scenarios.

In the field of Reproductive Endocrinology and Infertility some ultrasound and surgical skills are required for independent practice including transvaginal oocyte pick-up, hysterosalpingocontrastsonography (HyCoSy) and embryo-transfer. Considering advantages and disadvantages of both apprenticeship models (traditional training and simulator-based training), a blended approach which combines simulator-based training with didactic and more traditional training on real patients could be introduced. So far the use of simulation-based training has shown promising results in terms of moderate effects on patient outcomes and large effects on trainee behavior in the clinical setting. It could be useful especially in the initial part of a structured training for novices, enabling them to acquire basic skills and to reach a predefined level of performance in a safe and controlled environment, before applying the procedure to real patients.

INVITED SESSION

SESSION 41: MODERN FORMATS OF NURSE-PATIENT COMMUNICATION

29 June 2021 Stream 3 17:00 - 18:00

O-051 Telenursing within fertility

V. Peddie[1]

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Abstract text

Aberdeen Fertility Centre provides fertility investigation and treatment across the north east, highlands and islands (Orkney & Shetland) of Scotland, which includes rural and remote communities. Whilst the SARS-Cov-2 pandemic has accelerated the delivery of *Tele-fertility Nursing*, the National Health Service (NHS) in Grampian, Scotland launched both '*No Delays*' and '*Near Me*' platforms in 2019 to keep pace with the digital age and with the intention of improving the quality of patient care.

No Delays (series of video packages and virtual consultations) has revolutionised clinical pathways, providing individualised patient information from investigation to treatment, permitting email 'prescription' of digital postcards (personalised package of short videos introducing members of the team and explaining their role) that explain the fertility journey and management plan in detail, from investigation (ie., tubal evaluation), to treatment (ie., ovulation induction or IVF), and self-administration of medicines.

Thereafter, patients receive invitation to virtual '*Near Me*' consultation which can take place in the comfort of their own home whilst acknowledging flexibility in lifestyle (partner can remotely access consultation from workplace, including overseas).

Electronic patient records (EPR's) provide immediate access to complete, accurate and up to date clinical patient data, which, together with Electronic Consents compliment the tele-nursing experience through a complete library of consent forms, automatically allocated to patient and partner according to treatment type and personal circumstances: information being provided in video format to meet the requirements for informed consent. Forms are completed online, at home or in the 'virtual' clinic via computers, tablets or smartphones, with workflow automation tools allowing for digital signature/s. The complete digital package ensures patients have an early understanding of the process, therefore better prepared for face-to-face appointments and ensuring quality care delivered at point of contact.

The aims and objectives of '*Tele-fertility Nursing*' were to: (1) reinforce key information routinely provided at consultation which may not always be understood, (2) meet patient need and lifestyle, (3) increase patient choice, (4) save time and money by reducing unnecessary travel (often complicated by adverse weather conditions from rural communities), (5) avoid unnecessary time off work, and (6) reduce environmental impact of attended appointments (health miles and miles not travelled).

Whilst concern remains around the digital interface and nurse/patient relationship, preliminary evaluation of '*Near Me*' consultations and patient feedback - relative to electronic consent - suggests overall patient satisfaction; the above aims and objectives being met. However, full evaluation of the patient and fertility nurse experience of the 'digital fertility journey' is required.

O-052 Web-based applications and online patient portals to improve patient communication

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[1]*Guy's and St Thomas' Hospital, Assisted Conception Unit, London, United Kingdom*

SELECTED ORAL COMMUNICATIONS

SESSION 42: IMPROVING CONCEPTION BY REPRODUCTIVE SURGERY

29 June 2021 Stream 4 17:00 - 18:00

O-137 Salpingectomy versus neosalpingostomy in women with hydrosalpinx: a prospective cohort study with long-term follow-up

L. Yan[1,2], **C. Zhu**[1,2], **G. Liang**[1,2], **C. He**[1,2], **Y. Liang**[1,2], **X. Zhao**[1,2], **X. He**[1,2], **Y. Zhang**[1,2], **B.W. Mol**[3,4], **J.A.F. Huirne**[5], **J. Zhang**[1,2]

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[4]*Aberdeen Centre for Women's Health Research- University of Aberdeen, Department of Obstetrics and Gynecology, Aberdeen, United Kingdom ;*
[5]*Amsterdam Reproduction and Development Research institute- Amsterdam University Medical Centre, Department of Obstetrics and Gynecology, Amsterdam, New Zealand*

Study question: What is the comparative effectiveness between salpingectomy and neosalpingostomy in the treatment of bilateral severe hydrosalpinx?
Summary answer: In women with bilateral severe hydrosalpinx, salpingectomy combined with In Vitro Fertilization (IVF) treatment resulted in a higher cumulative live birth rate than neosalpingostomy.
What is known already: Hydrosalpinx accounts for 25% to 35% of female subfertility and has a negative effect on pregnancy rates in women who undergo IVF. So far salpingectomy before in vitro fertilization treatment has been suggested for the treatment of hydrosalpinx in order to improve the chance of a live birth. Previous studies have reported a pooled live birth rate of 25% through natural conception after neosalpingostomy and an ongoing pregnancy rate of 55.8% after salpingectomy with IVF. Direct comparison of the cumulative live birth rate after salpingectomy versus neosalpingostomy, both followed by IVF is lacking.
Study design, size, duration: We performed a single center, prospective comparative cohort study in the International Peace Maternity and Child Health Hospital affiliated to Shanghai Jiao Tong University School of Medicine, China. We studied women diagnosed with tubal subfertility with bilateral hydrosalpinx between January 2005 and December 2012. Based on a shared decision approach, they had bilateral salpingectomy or neosalpingostomy followed by IVF. They were annually followed up until July 2020 for the occurrence of live birth.
Participants/materials, setting, methods: Out of 113 women, 55 had bilateral salpingectomy and 58 had bilateral neosalpingostomy. Primary outcome

was cumulative live birth rate, defined as the cumulative birth rate of the first living neonate through either natural conception or in vitro fertilization. Both intention-to-treat (ITT) and per-protocol (PP) analysis were processed. Cox proportional hazards regression model with potential variables was performed to identify predictors of successful live birth.

Main results and the role of chance: Baseline characteristics were comparable between two groups. There were 42 live births in the salpingectomy and 36 in the neosalpingostomy group. When the result of IVF was incorporated (55/55 in salpingectomy group and 25/58 in neosalpingostomy group underwent IVF), salpingectomy resulted in a higher cumulative live birth rate (85.3% vs 76.0%; hazard ratio of the whole survival curve, 2.18; 95% CI, 1.37 - 3.45; P = 0.001), a lower risk of ectopic pregnancy (1.8% vs 20.7%; risk ratio, 0.07; 95% CI, 0.01 - 0.57; P = 0.013), and a shorter time to live birth (19 [14,27] versus 36 [17,76] months, P = 0.001).The number of live birth rates after natural conception was 0% (0/55) in the salpingectomy group and 28% (16/58) in neosalpingostomy group.The results of PP analyses were comparable with the ITT analyses apart from the biochemical pregnancy rate and the overall live birth rate, they were higher (the former: 76% (42/55) versus 58% (29/50), P = 0.045; the latter: 76% (42/55) versus 56% (28/50), P = 0.027) in salpingectomy group.

Limitations, reasons for caution: This is an observational study. The small sample size along with the data was obtained from a cohort study in a single center.

Wider implications of the findings: In women with confirmed bilateral severe hydrosalpinx, salpingectomy followed by IVF results in a higher cumulative live birth rate and decreases the risk of EP as compared to neosalpingostomy. However, neosalpingostomy is the only option to achieve a live birth by natural conception that should be discussed with patients preoperatively.

Trial registration number: not applicable

O-138 Reproductive performance of women with and without intrauterine adhesions following recurrent dilatation and curettage for miscarriage: long-term follow-up of a randomized controlled trial

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Study question: Are the long-term reproductive outcomes following recurrent dilatation and curettage (D&C) for miscarriage in women with identified and treated intrauterine adhesions (IUAs) comparable to women without IUAs.

Summary answer: Reproductive outcomes in women with identified and treated IUAs following recurrent D&C for miscarriage are impaired compared to women without IUAs.

What is known already: The Prevention of Adhesions Post Abortion (PAPA) study showed that application of auto-crosslinked hyaluronic acid (ACP) gel, an absorbable barrier in women undergoing recurrent D&C for miscarriage resulted in a lower rate of IUAs, 13% versus 31% (relative risk 0.43, 95% CI 0.22 to 0.83), lower mean adhesion score and significant less moderate to severe IUAs. It is unclear what the impact is of IUAs on long-term reproductive performance.

Study design, size, duration: This was a follow-up of the PAPA study, a multicenter randomized controlled trial evaluating the application of ACP gel in women undergoing recurrent D&C for miscarriage. All included women received a diagnostic hysteroscopy 8–12 weeks after randomization to evaluate the uterine cavity and for adhesiolysis if IUAs were present. Here, we present the reproductive outcomes in women with identified and treated IUAs versus women without IUAs, 46 months after randomization.

Participants/materials, setting, methods: Between December 2011 and July 2015, 152 women with a first-trimester miscarriage with at least one previous D&C, were randomized for D&C alone or D&C with immediate intrauterine application of ACP gel. Participants were approached at least 30 months after randomization to evaluate reproductive performance, obstetric and neonatal outcomes and cycle characteristics. Main outcome was ongoing pregnancy. Outcomes of subsequent pregnancies, time to conception and time to live birth were also recorded.

Main results and the role of chance: In women pursuing a pregnancy, 14/24 (58%) ongoing pregnancies were recorded in women with identified and treated IUAs versus 80/89 (90%) ongoing pregnancies in women without IUAs odds ratio (OR) 0.18 (95% CI 0.06 to 0.50, P-value <0.001). Documented live birth was also lower in women with IUAs; 13/24 (54%) with versus 75/89 (84%) without IUAs, OR 0.22 (95% CI: 0.08 to-0.59, P-value 0.004). The median time to conception was 7 months in women with identified and treated IUAs versus 5 months in women without IUAs (hazard ratio (HR) 0.84 (95% CI 0.54 to 1.33)) and time to conception leading to a live birth 15 months versus 5.0 months (HR 0.54 (95% CI: 0.30 to 0.97)). In women with identified and treated IUAs, premature deliveries were recorded in 3/16 (19%) versus 4/88 (5%) in women without IUAs, P-value 0.01. Complications were recorded in respectively 12/16 (75%) versus 26/88 (30%), P-value 0.001. No differences were recorded in mean birth weight between the groups.

Limitations, reasons for caution: In the original PAPA study, randomization was applied for ACP gel application. Comparing women with and without IUAs is not in line with the randomization and therefore confounding of the results cannot be excluded. IUAs, if visible during routine hysteroscopy after randomization were removed as part of the study protocol.

Wider implications of the findings: As IUAs have an impact on reproductive performance, even after hysteroscopic adhesiolysis, primary prevention is essential. Expectative and medical management should therefore be considered as serious alternatives for D&C in women with a miscarriage. In case D&C is necessary, application of ACP gel should be considered.

Trial registration number: Netherlands Trial Register NTR 3120.

O-139 Complete removal of uterine septum tissue as a wedge, an alternative method for patients suffering from recurrent pregnancy loss or infertility, a retrospective cohort study

F. Razzaghi Kashani[1], R. Zargham[1], S. Amirajam[1], H. Jadda[2], S. Razi[1], A. Jadda[1]

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[2]Avicenna Research Institute, Dept. of Embryology, Tehran, Iran

Study question: Is hysteroscopic wedge septectomy (HWS) an effective and safe method for reducing the risk of miscarriage and improving the reproductive outcome in patients with recurrent pregnancy loss or infertility history?

Summary answer: HWS is a safe and effective method for RPL and infertility cases with statistically significant improvement in pregnancy chances and reproductive outcomes.

What is known already: With regard to the persisting uncertainty around the effectiveness of septum resection in recurrent miscarriage and infertility cases, there may be alternative methods to better address the pathophysiology of septum. There are different explanations for the poor reproductive performance with uterine septum: poor vascularisation of a highly fibrous implantation site, low sensitivity of endometrial receptors covering the septa, its "myoma-like" composition, and finally higher uterine vascular resistance. Complete removal of this abnormal tissue rather than just incising it may not only enhance challenging the pathogenesis but also expand the endometrial volume, an objective parameter by which to predict endometrial receptivity.

Study design, size, duration: In this retrospective cohort study, 214 consecutive patients, aged 33.3±4.8, diagnosed with a septate uterus based on ESHRE classification who had been under HWS between April 2017 and January 2020 due to recurrent miscarriage or at least one failed embryo transfer, met the enrollment criteria. With 11 to 36 months follow up, gathering of follow up data was managed between August till the end of Nov 2020, when the last new information was included.

Participants/materials, setting, methods: Patients with a history of RPL or at least one failed ET who were diagnosed as septate uterus by 2D, 3D, or hysteroscopy have been under HWS in a tertiary infertility and recurrent abortion treatment/educational setting. Those with BMI≥32, day 3 FSH≥13 mIU/mL, acquired or hereditary thrombophilia, thyroid disease, and myomatous uterus were excluded. HWS's goal was to remove the septum as a wedge, cutting with 7Fr scissors, in its entirety as much as possible.

Main results and the role of chance: 39 patients who experienced 1 to 8 failed ET and 175 with 2 to 10 miscarriages, were enrolled in the study. The

average septum size based on the depth of the removed wedge was 1.73±0.86 cm. There was an increase of 1.68±0.9 cm in uterine depth and 2.28±0.6 ml in uterine capacity measured by uterine sound and inflation of 8F Folley catheter balloon inside the cavity, respectively. The procedure took 35.75±8.7 minutes. Intraoperative, postoperative, or late complications during the next pregnancies were not reported. 7 patients (17.9%) in failed ET group, conceived spontaneously, before another embryo transfer attempt. Embryo transfer in the remaining 32 cases resulted in 25 (78.1%) clinical pregnancies. 2 miscarried (6.2%), 5(15.6%) are ongoing after 20 weeks of gestation and 25 (78.1%) have resulted in live births. Among 126 clinical pregnancies in RPL group, 16 patients (12.6%) experienced another miscarriage; 6%, 11.3%, and 25% in patients with a previous history of 2, 3, and 4 or more miscarriages, respectively. There was a significant drop in odds of post-procedure miscarriage from 22.7% to 6% (p:0.005) and from 27.8% to 11.3% (p:0.27) with 2 and 3 miscarriage history, respectively. This reduction was not significant with more than 3 losses.

Limitations, reasons for caution: We acknowledge the inherent limitations of this retrospective observational study, confining direct inferences. Our goal is to encourage future prospective studies to compare the effectiveness of different methods of hysteroscopy with or without involving the removal of septal tissue. An RCT comparing metroplasty vs expectant management seems infeasible, though.

Wider implications of the findings: Our findings suggest that timely removal of the uterine septal tissue with hysteroscopy will result in favorable reproductive outcomes in patients with RPL and/or infertility. Also, a history of a normal term pregnancy before subsequent successive losses does not rule out the uterine septum and calls for a thorough assessment.

Trial registration number: not applicable

O-140 Uterine septum: clinical implications on fertility and obstterics outcomes. A systematic review and meta-analysis

G. Spagnol[1], G. Bonaldo[1], M. Marchetti[1], A. Vitagliano[1], A.S. Laganà[2], M. Scioscia[3], A. Andrisani[1], G. Ambrosini[1], D. D'Antona[1], A. Riva[1], C. Saccardi[1], M. Noventa[1]

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Study question: How does the septate uterus and his metroplasty influence pregnancy rate (PR), live birth rate (LBR), spontaneous abortion rates (SA) and preterm labour rates (PL)?

Summary answer: Uterine septum is associated with a poor reproductive outcome. Metroplasty reduce the rate of SA but non-conclusive evidence can be extrapolated about PR and PL.

What is known already: Different studies evaluated the correlation between uterine septum and reproductive outcomes. On one hand, studies reported its association with poor obstetrics outcomes. On the other hand, recent studies raised doubts about the effectiveness of septum metroplasty to improve reproductive outcomes, although recent position papers continue to propose metroplasty in patients with a septate uterus and a history of infertility or miscarriages. Debate is still ongoing on reproductive outcomes of uterine septum on infertile patients and especially on patients with recurrent miscarriage, leading to an unanswered question whether or not these women should be treated.

Study design, size, duration: Systematic review and meta-analysis of published studies that evaluated the clinical impact of uterine septum and its metroplasty on reproductive and obstetrics outcomes. The meta-analysis included study with infertile patients or patients with a history of recurrent miscarriage. Searches were conducted using the following search terms: uterine septum, septate uterus, metroplasty, pregnancy rate, live birth rate, spontaneous miscarriage, infertility, preterm delivery. Primary outcomes were PR and LBR. Secondary outcomes were SA and PL.

Participants/materials, setting, methods: The meta-analysis was written following the PRISMA guidelines. Fifty-nine full-text articles were preselected based on title and abstract. Endpoints were evaluated in three subgroups: 1) infertile/recurrent miscarriage patients with septum versus no septum 2) infertile/recurrent miscarriage patients with treated versus untreated septum 3) infertile/recurrent miscarriage patients before-after septum removal. Odds-ratios (OR) with 95% confidence intervals (CI) were calculated for outcome measures. Random-effect meta-analysis was performed and a p-value less than 0.05 was considered statistically significant.

Main results and the role of chance: Data from 37 articles were extracted. In the first subgroup (10 studies), a lower PR and LBR were associated with septate uterus vs. controls, respectively (OR 0.39, 95% CI 0.26 to 0.58; p<0.000; low-heterogeneity and OR 0.21, 95% CI 0.12 to 0.39; p<0.0001; small-heterogeneity) and a higher proportion of SA and PL was associated with septate uterus vs. controls, respectively (OR 4.17, 95% CI 2.83 to 6.15; p<0.000; moderate-heterogeneity and OR 2.18, 95% CI 1.27 to 3.76; p=0.005; low-heterogeneity). In the second subgroup (8 studies), PR and PL were not different in removed vs. unremoved septum, respectively (OR 1.10, 95% CI 0.49 to 2.49; p=0.82; moderate heterogeneity and OR 0.44, 95% CI 0.18 to 1.08; p=0.08;low-heterogeneity) and a lower proportion of SA was associated with removed vs. unremoved septum (OR 0.40, 95% CI 0.17 to 0.95; p=0.001; substantial-heterogeneity). In the third subgroup (19 studies), the proportion of LBR was higher after the removal of septum (OR 49.58, 95% CI 29.93 to 82.13; p<0.0001; moderate-heterogeneity) and the proportion of SA and PL was lower after the removal of septum, respectively (OR 0.02, 95% CI 0.02 to 0.04; p<0.000; moderate-heterogeneity and OR 0.05, 95% CI 0.03 to 0.08; p=<0.000; low-heterogeneity).

Limitations, reasons for caution: The present meta-analysis is limited by the observational design of included studies because, in literature, there are no prospective randomized controlled trials (RCTs). In the second and third subgroup of analysis clinical heterogeneity within and between studies represents another limitation.

Wider implications of the findings: The results of this meta-analysis confirm the detrimental effect of uterine septum on PR, LBR, SA and PL. Its treatment seems to reduce the rate of SA. Metroplasty should still be considered as good clinical practice in patients with a history of infertility and recurrent abortion.

Trial registration number: Not applicable

ESHRE 2021 / Oral presentations

INVITED SESSION

SESSION 43: MEN AS CANARIES: HOW EPIGENETICS & GENETICS REFLECTS THE WORLD WE LIVE IN

30 June 2021 Stream 1 08:30 - 09:30

O-053 Early life factors that may influence adult male reproductive

R. Hart[1]

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Abstract text

This presentation will provide a brief overview of testicular development and will describe a critical period of development at approximately 8-14 weeks of gestation, when the testicle may be vulnerable to external influences, potentially having a negative effect on subsequent development. The talk with then describe the testicular dysgenesis syndrome hypothesis, proposed by Niels Skakkebæk, and using the presenters work within the Western Australian (Raine) Cohort will go onto demonstrate how early life exposures may influence mature testicular function. Worryingly, the presentation will describe how adolescent features of early metabolic disturbance within the Raine Cohort are already having a determinantal effect on the reproductive function of these men in late adolescence, many years before the majority are seeking paternity.

O-054 From the global to the molecular: male reproduction under attack

C. Barratt[1]

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INVITED SESSION

SESSION 44: EMBRYO SCREENING FOR POLYGENIC TRAITS: THE GOOD, THE BAD AND THE UGLY

30 June 2021 Stream 2 08:30 - 09:30

O-055 Why we should be testing embryos for polygenic traits

J. Savulescu[1]

[1]*University of Oxford, Faculty of Philosophy, Oxford, United Kingdom*

Abstract text

The past few years have brought significant breakthroughs in understanding human genetics. This knowledge has been used to develop 'polygenic scores' (or 'polygenic risk scores') which provide probabilistic information about the development of polygenic conditions such as diabetes or schizophrenia. They are already being used in reproduction to select for embryos at lower risk of developing disease. The world's first baby, Aurea, has been born after being selected on the basis of a Genomic Health Index score provided by Genomic Prediction (https://www.youtube.com/watch?v=HE5ADe7BgdM&ab_channel=GenomicPrediction).

I argue for the use of this technology not only for the selection of embryos at lower risk of disease but for the selection of advantageous non-disease traits.

Currently, the use of polygenic scores for embryo selection is subject to existing regulations concerning embryo testing and selection. Existing regulatory approaches include 'disease-based' models which limit embryo selection to avoiding disease characteristics (employed in various formats in Australia, the UK, Italy, Switzerland and France, among others), and 'laissez-faire' or 'libertarian' models, under which embryo testing and selection remain unregulated (as in the USA). With Sarah Munday, I have introduced a novel 'Welfarist Model' which limits embryo selection according to the impact of the predicted trait on well-being. We compare the strengths and weaknesses of each model as a way of regulating polygenic scores. Polygenic scores create the potential for existing embryo selection technologies to be used to select for a wider range of predicted genetically influenced characteristics including continuous traits. Indeed, polygenic scores exist to predict future intelligence, and there have been suggestions that they will be used to make predictions within the normal range in the USA in embryo selection. We examine how these three models would apply to the prediction of non-disease traits such as intelligence. The genetics of intelligence remains controversial both scientifically and ethically. This paper does not attempt to resolve these issues. However, as with many biomedical advances, an effective regulatory regime must be in place as soon as the technology is available. If there is no regulation in place, then the market effectively decides ethical issues.

O-056 Evaluating the utility of screening human IVF embryos with polygenic risk scores for complex diseases

S. Carmi[1], **D. Backenroth**[1], **A. Green**[1], **O. Weissbrod**[2], **O. Zuk**[3], **T. Lencz**[4]

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[3]*The Hebrew University of Jerusalem, Statistics, Jerusalem, Israel ;*
[4]*Zucker School of Medicine at Hofstra/Northwell, Psychiatry and Molecular Medicine, Hempstead- NY, U.S.A.*

Study question: It is now feasible to screen human IVF embryos with "polygenic risk scores" for predicting complex disease risk. What is the expected risk reduction?

Summary answer: Under some conditions, prioritizing embryos based on polygenic risk scores can lead to substantial disease risk reductions. However, only excluding high-risk embryos is less effective.

What is known already: Recent genetic studies have identified numerous mutations associated with complex diseases, leading to the development of accurate polygenic risk scores (PRSs) for disease risk prediction. Given that genomes of human IVF embryos can now be sequenced with relative ease, it has become technically feasible to use PRSs for prioritization of embryos for transfer. Clearly, such use is associated with ethical and social concerns, from inequality to eugenics. Nevertheless, polygenic embryo screening is already offered to consumers, with little research so far on expected outcomes. Our previous evaluation of screening IVF embryos for polygenic traits showed little current utility.

Study design, size, duration: This is a theoretical/computational study based on statistical genetics theory and simulations.
Participants/materials, setting, methods: We used the liability threshold model to estimate the disease risk given the PRS. We considered screening for a single disease (with known prevalence and PRS accuracy), and assumed that n viable embryos are available. We calculated the risk of the child given these parameters and the prioritization strategy, either when parents are random or when their disease status is known. We also used simulations based on genomic data from a schizophrenia case-control study.
Main results and the role of chance: We modeled the disease risk of a hypothetical future child when the PRSs of embryos are used for prioritization, relative to random selection. When selecting an embryo at random among those who do not have an extremely high risk (typically, top 2% of the PRS distribution), the relative risk reduction (RRR) is limited, and is under 10% for currently realistic scenarios. In contrast, selecting the lowest risk embryo for implantation results in substantial RRRs of ~20-50% already with n=5 embryos and realistic disease parameters. For example, the RRR for schizophrenia is >40% with current PRSs, a result we validated with simulated genomes of parents and children based on genotypes from a schizophrenia study. When one of the parents is known to be affected, selecting the lowest risk embryo out of n=5 may restore the risk of the future child to nearly normal levels.
Limitations, reasons for caution: Our analytical modeling is based on several simplifying assumptions regarding the dependence of the risk on the PRS and the accuracy of the PRS. Further, the estimated risk reductions depend on the availability of n=5 embryos that could lead to a live birth.
Wider implications of the findings: Under some conditions, prioritizing embryos for transfer based on polygenic risk scores could lead to substantial disease risk reductions. However, predicted outcomes vary considerably with prioritization strategies and disease and PRS parameters. The emerging ethical and social concerns and the challenges in communicating these results need to be urgently discussed.
Trial registration number: Not applicable

POSTER DISCUSSION

SESSION 45: MALE AND FEMALE FERTILITY PRESERVATION POSTER DISCUSSIONS

30 June 2021 Stream 3 08:30 - 09:30

P-437 The ovaries of transgender men indicate effects of high dose testosterone on the primordial and early growing follicle pool

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[1]University of Edinburgh, reproductive biology, Edinburgh, United Kingdom ; [2]Queen Elizabeth University Hospital, Gynaecology, Glasgow, United Kingdom ; [3]Royal Infirmary Edinburgh, Gynaecology, Edinburgh, United Kingdom ; [4]Spire Thames Valley Hospital, Gynaecology, London, United Kingdom

Study question: Does high-dose testosterone therapy affect the stage distribution, morphological health and DNA damage repair capacity of human ovarian follicles and their survival *in vitro*?
Summary answer: Testosterone exposure is associated with reduced follicle growth activation, reduced follicle health and increased DNA damage: these further deteriorate after six days of culture.
What is known already: Androgens have diverse actions within the ovary, however, there is a lack of information regarding the long-term effects of high-dose testosterone on ovarian function and reproductive potential. Cumulus-oocyte complexes recovered from transgender men have been successfully matured *in-vitro* but little is known regarding the impact of this gender affirming endocrine therapy on the primordial follicle pool.
Study design, size, duration: Whole ovaries were obtained from four transgender men aged 25-36 years with informed consent at oophorectomy. All patients had received 1000mg testosterone undecanoate intramuscularly at 12-16 week intervals for a minimum of 4 years pre-operatively. Cortical tissues were dissected into small pieces (≈1x1x0.5mm) and either immediately fixed for histological analysis or cultured for 6 days. Testosterone-treated ovaries were compared to cortical biopsies from age-matched healthy women obtained at caesarean section (n=4, age 26-36).
Participants/materials, setting, methods: Follicle number, classification of developmental stage and morphology were evaluated by histological analysis of ovarian cortical tissue from day 0 and 6 days post culture. Immunohistochemical analysis included γH2AX as a marker of DNA damage, and meiotic recombination 11 (MRE11), ataxia telangiectasia mutated (ATM) and Rad51 as DNA repair proteins. A total of 3802 follicles from testosterone exposed and 878 from control ovaries were analysed.
Main results and the role of chance: At day 0 (D0), transgender tissue had a higher proportion of non-growing follicles (92.7±1.7%) compared to control (85.4±6.2%, $p<0.05$) but a lower proportion of morphologically healthy follicles (non-growing 59%, primary 61%, secondary 36%; vs 83%, 75%, 80% in controls, all $p<0.005$). After 6 days in culture, the proportion of growing follicles increased (51.3% vs 46.5%) but follicle health further declined (all stages $p<0.005$).

DNA damage was assessed by expression of γH2AX. At D0, the proportion of oocytes showing DNA damage was significantly higher in transgender non-growing follicles (48.1±12.5%, vs 12.3±0.25%, $p<0.005$). After culture, γH2AX expression increased in both transgender ($p<0.005$) and controls ($p<0.005$) but remained higher in transgender oocytes (non-growing 72.2%, primary 71.7% vs 27.3%, 46.2%, all $p<0.05$).

At D0, there was no difference in expression of DNA repair enzymes ATM and RAD51 between transgender and control oocytes, and increased expression of MRE11 in control non-growing follicles ($p<0.05$). Post-culture, there was a significant increase in ATM expression in transgender non-growing oocytes compared to control (98.5% vs 77.8%, $p<0.05$) and a less marked decline in RAD51 expression($p<0.05$). The expression of MRE-11 in control non-growing oocytes dramatically declined (100% to 58.2%, $p<0.05$), unlike in transgender tissue where expression was comparable to D0.
Limitations, reasons for caution: A large number of follicles have been analysed, but only from a small number of ovaries. DNA damage at D0 and after 6 days of culture may not reflect DNA damage and repair capacity at later stages of follicle growth. The effect of duration of testosterone treatment was not investigated.
Wider implications of the findings: These data indicate that high circulating concentrations of testosterone have previously unrecognised effects on the primordial and small-growing follicles of the ovary. These results may have implications for transgender men receiving gender-affirming therapy prior to considering pregnancy or fertility preservation measures.
Trial registration number: n/a

P-444 Presence of pharmacological inhibitors of the PI3K/PTEN/Akt and mTOR signalling pathways during cryopreservation and organotypic cultures of murine ovaries limits early primordial follicle depletion

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Study question: Which signalling pathways are implicated in primordial follicle activation induced by cryopreservation and/or organotypic culture? Is it possible to limit this activation through pharmacological inhibitors?
Summary answer: Our findings provide support for the hypothesis that mTOR and PI3K inhibitors might represent an attractive tool to delay cryopreservation- and culture-induced primordial follicle activation.
What is known already: Cryopreservation of ovarian tissue containing immature primordial follicles followed by auto-transplantation (OTCTP) is the only option available to preserve the fertility of prepubertal patients or patients requiring urgent therapy for aggressive malignancies. However, a major obstacle in this process is follicular loss immediately after grafting, possibly due to slow neovascularization, apoptosis and/or massive follicular recruitment. *In vitro* and *in vivo* studies indicate that the PI3K/PTEN/Akt and mTOR signalling pathways are involved in follicle activation. The transplantation process seems to be the

major cause of primordial follicle activation after OTCTP but information about how cryopreservation itself impacts follicle activation is sparse.
Study design, size, duration: Whole murine ovaries (4-8-weeks old) were cryopreserved by slow freezing and exposed to LY294002 (a powerful PI3K inhibitor) or rapamycin (a specific mTOR inhibitor) during cryopreservation and/or organotypic *in vitro* culture for a 24 h or 2 days.
Participants/materials, setting, methods: Western Blot and immunofluorescence analyses were used to determine the activation of PI3K/PTEN/Akt and mTOR signalling pathways in murine ovaries cryopreserved and/or organotypically cultured with/without inhibitors.Follicles were quantified according to their maturation degree on H&E stained histological sections.
Main results and the role of chance: Ratio of phosphorylated Akt or rps6 to total proteins (p-Akt/Akt and p-rps6/rps6) was increased in slow-frozen murine ovaries compared to control fresh ovaries, indicating an activation of the PI3K/PTEN/Akt and mTOR signalling pathways. The use of pharmacological inhibitors of follicle signalling pathways (LY294002 (25µM) and rapamycin (1µM)) during the cryopreservation process decreased p-Akt/Akt and p-rps6/rps6 ratios. *In vitro* organotypic culture for 24 h increased only the activation of the PI3K/PTEN/Akt pathway, as shown by increased p-Akt/Akt ratio in fresh ovaries cultured for 24 h compared to fresh non-cultured ovaries. This activation can be counteracted by cryopreservation of murine ovaries with rapamycin followed by *in vitro* culture for 24 h in the presence of LY294002. Follicle density quantifications indicated that when cryopreserved ovaries were maintained in culture for 2 days, a decrease of primordial follicle density concomitant with an increase of secondary and more mature follicles were found in comparison to slow-frozen/thawed ovaries without culture. Supplementation of the culture medium with LY294002 and rapamycin for 24 h or 2 days preserved primordial follicle densities compared to ovaries cultured without inhibitors.
Limitations, reasons for caution: This study is an *in vitro* study using murine ovaries. To analyze the efficiency of LY294002 and rapamycin to limit cryopreservation and transplantation induced follicle recruitment, these inhibitors should be tested in an *in vivo* model. Furthermore, these findings will need to be confirmed with human samples.
Wider implications of the findings: We showed for the first-time that the sequential use of pharmacological inhibitors, rapamycin during the slow freezing process followed by organotypic culture supplemented with LY294002, is effective to limit early primordial follicle depletion.
Trial registration number: /

P-450 Effects of rheumatoid arthritis and methotrexate therapy on ovarian reserve in infertile women

G. Vlasova[1], S. Perminova[1]

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Study question: Do patients with infertility and rheumatoid arthritis (RA) treated with methotrexate (MTX) have ovarian reserve alterations?
Summary answer: Women with infertility and RA treated with MTX were found to have statistically significant decrease of ovarian reserve.
What is known already: Rheumatoid arthritis (RA) is one of the most prominent inflammatory diseases affecting women of child-bearing age [Brouwer J. et al, 2014]. RA and its treatment may interfere with the female reproductive physiology. The vast majority of patients with RA are treated with methotrexate (MTX) which is a folate antagonist that inhibits DNA synthesis. MTX, which is the anchor drug in RA, targets actively proliferating cells including the oocytes and granulosa cells which may impair the ovarian reserve [Min Tun Kyaw et al, 2020].
Study design, size, duration: A prospective case-control study that enrolled 72 female patients with infertility was conducted in the 2-year time period of September 2018 to October 2020.
Participants/materials, setting, methods: The main group comprised 32 patients with infertility and RA;

the control group consisted of 40 women with infertility only. Patients with RA were stratified into subgroups based on whether or not they received MTX.

To investigate ovarian reserve measurement of serum anti-Müllerian hormone (AMH) was used. The level of AMH was evaluated concerning RA duration and activity, as well as the age at initiation of MTX therapy, dosage, and treatment duration.

Main results and the role of chance: The mean age of the study population was 36±3 years. The duration of RA was 4 [3;11] years. The low disease activity based on DAS28-ESR (disease activity score based on 28 joints using the erythrocyte sedimentation rate) prevailed(56.2%).

In the main group 19(59.4%) women received MTX therapy. The MTX dosage was 15 [15;20]mg /wk, the duration of MTX therapy by the day of inclusion in the study was 18.7[1;15]months.

The AMH level was significantly lower in the main group (2.1 n /ml vs 2.73ng /ml, p=0.043). The number of patients with decreased ovarian reserve (AMH level<1.0ng/ml) significantly prevailed in the group of patients with RA (25% vs 5%, p=0.015).

When assessing the AMH level in patients with RA who received MTX (n=19) and patients in the control group, there was a tendency towards a decrease in the indicator in the first subgroup, but no statistically difference was found (p=0.074).

Correlation analysis of the dependence of AMH level on the patient age showed the most significant decrease in AMH in the patients with RA receiving MTX compared to the patients with RA who did not, and compared to all patients with RA regardless of the therapy received (rs=-0.563)(p <0.05).
Limitations, reasons for caution: The lack of statistically significant data in certain cases may be due to the small sample size.
Wider implications of the findings: RA and MTX administration are associated with a significant decrease in AMH levels. The age of initiation of the therapy is negatively correlated with the AMH level. In this regard, patients with already compromised reproductive function who are planning to receive MTX should be advised to preserve the genetic material.
Trial registration number: 567890

P-461 Pre-selected for an award: A 16-year bicentric retrospective analysis of ovarian tissue cryopreservation (OTC) in paediatric patients: indications, results and outcome

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Study question: What is the outcome of ovarian tissue cryopreservation (OTC) in paediatric patients from the beginning of its setting in two different French centres?
Summary answer: In our cohort of 75 paediatric patients who underwent OTC, the mean age, malignancy rate and survival rate were 9.7 years, 70.7% and 77.3% respectively.
What is known already: Cancer treatments of last decades improve the survival rate of children and adolescents;

however chemo- and radiotherapy result in gonadal damage leading to acute ovarian failure and sterility. The preservation of fertility is now an integral part of care of children requiring gonadotoxic treatments.

Currently OTC represents the only possibility of preserving the potential fertility in prepubertal girls. OTC is an effective fertility preservation option which allows long-term storage of primordial follicles, subsequent transplantation restores endocrine function and fertility. The efficacy of these techniques is well-demonstrated within adult population but the data are poor for paediatric patients.

Study design, size, duration: This is a retrospective study of OTC practice of two French centres from January 2004 to May 2020.
Participants/materials, setting, methods: A total of 75 patients from paediatrics units underwent cryopreservation of ovarian tissue before gonadotoxic therapy for malignant or benign diseases. The ovarian cortex was cut into fragments and the number of follicles per square millimeter was evaluated histologically. The long-term follow-up includes survival rate, hormonal and fertility status.
Main results and the role of chance: The mean age at OTC of 75 patients was 9.7 years [0.2 – 20], 32% were postpubertal. 53 had malignant disease and 22 had non-malignant disease. The most frequent diagnoses in this cohort included acute leukemia, hemoglobinopathies and neuroblastoma. Indication for OTC was stem cell transplantation for 78.7% (n=59) girls.

A third of each ovary was collected for 62,7% (n=47) patients, a whole ovary for 33,3% (n=25) patients and a third of one ovary alone for 4,0% (n=3) patients. An average of 17 fragments [5-35] per patient was cryoconserved. A correlation was found between age and the number of fragments ($p < 0.001$). More fragments were obtained from partial bilateral harvesting than from whole ovary harvesting ($p<0.05$). Histological analysis of ovarian tissue showed a median of 6.0 primordial follicles/mm2 [0.0–106.5] and no malignant cells were identified. A negative correlation was found between age and follicular density ($p<0.001$).

Median post-harvest follow-up was 92 months [1–188]: 17 girls had died, 12 were still treated for their pathology and 46 were in complete remission. Of all patients, 29 have been subject to hormonal status evaluation and 26 were diagnosed with premature ovarian insufficiency ($p<0.001$). One patient had undergone thawed ovarian tissue transplantation.
Limitations, reasons for caution: This study is a retrospective analysis. The cohort was not compared with a control group who did not undergo OTC or with an adult population. Furthermore, many of these girls are still young and do not intend to use the transplantation of thawed ovarian tissue yet.
Wider implications of the findings: OTC should be proposed to all girls with high risk of developing premature ovarian insufficiency following gonadotoxic therapies in order to give them the possibility of fertility and endocrine restoration.
Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 46: CURRENT CHALLENGES IN UTERINE DISORDERS

30 June 2021 Stream 1 10:00 - 11:30

O-141 Mapping of SARS-CoV-2-associated receptors and proteases mRNA in human endometrium during natural and stimulated cycles

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[4]CHU and University of Montpellier, Department of Reproductive Medicine, Montpellier, France

Study question: Covid-19 pandemic has significantly affected the assisted reproductive technology (ART) practice. Understanding whether SARS-CoV-2 could infect endometrial tissues during ART is crucial for risk mitigation
Summary answer: Analyses of gene expression profiles of SARS-CoV-2 host entry candidates from microarray data suggest that endometrium should be considered as potential target for SARS-CoV-2 infection.
What is known already: Very few studies analyzed the gene expression profiles of SARS-CoV-2-associated receptors and proteases, mainly focusing on *ACE2* and *TMPRSS2* expression, resulting incomplete knowledge in different specimens from female genital tract. However, no studies have analyzed the potential impact of controlled ovarian stimulation (COS) protocols during ART procedure on the endometrial gene expression profiles of SARS-CoV-2-associated receptors and proteases
Study design, size, duration: To address this question, we retrospectively examined the gene expression profile of SARS-CoV-2-associated receptors and proteases in endometrial biopsies of a cohort of ART candidates using Affymetrix microarray data
Participants/materials, setting, methods: Human endometrial tissue under natural (n=62) and COS cycles (n=42) were analyzed. A focus was particularly made on the renin-angiotensin system relates genes with a prominent role in the virus infection, and gene expression levels of receptors and proteases closely related to SARS-CoV-2 infectionwas also studied.
Main results and the role of chance: Using our large cohort of endometrial samples, we reported a high prevalence of genes related to the ACE2 pathway, including *AGT*, *AGTR1*, *ANPEP*, *CTSA*, *ENPEP*, *LNPEP*, *MME*, *NLN*, *THOP1*, *BSG* and *CTSL* during both phases(early- and mid-secretory phase), and mainly during the mid-secretory phase for *ACE2*. The highest signal intensities were found for *CTSA*, *LNPEP*, *MME*, *NLN*, *BSG* and *CTSL*. The most representative of dual coexpression of SARS-CoV-2-associated receptor and protease in endometrium was *BSG-CSTL* and *BSG-CTSA*. It s also important to note high variation of SARS-CoV-2 receptors inter-patients under natural cycle.Globally, the impact of COS on endometrial gene expression profile of SARS-CoV-2-associated receptors and proteases of non Covid-19 patients is low, suggesting no additional potential risks of SARS-CoV-2 infection during stimulated ART procedure compared with natural cycles.
Limitations, reasons for caution: Analyses of Affymetrix microarray gene expression data were performed in non-COVID-19 patients. Whether the SARS-CoV-2 infection changes the endometrial gene expression profile of SARS-CoV-2-associated receptors and proteases is under investigation
Wider implications of the findings: Specimens from female genital tract may be considered as potential targets for SARS-CoV-2.
Trial registration number: not applicable

O-142 COVID19-free endometrium: Undetectable viral RNA in endometrial biopsies from positive symptomatic SARS-CoV-2 women

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[4]Instituto de investigación sanitaria La Fe, Reproductive Medicine Research Group, Valencia, Spain ;
[5]IVIRMA Rome, Gynecology, Rome, Italy

Study question: Does SARS-CoV-2 infect the endometrial tissue in women with coronavirus disease 2019 (COVID-19)?
Summary answer: Symptomatic women with COVID-19 report no presence, in the short term, of viral RNA from SARS-CoV-2 in the endometrium.
What is known already: The recent emergence of COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has not allowed yet to establish putative relations between this disorder and other existing pathologies. It is the case with fertility problems and the reproductive organs, including a possible endometrial disorder caused by the virus. Thus, an important step is to elucidate the viral presence in different human tissues to improve diagnostics, prevention and/or treatment. The hypothesis of the possible infection of the endometrial tissue comes from the described expression of ACE2 protein in the human endometrium, mainly in stromal cells during the secretory phase.
Study design, size, duration: 15 endometrial biopsies from symptomatic and hospitalized women with COVID-19 were collected. Endometrial samples were obtained from August to November 2020 at the Hospital Universitari i Politècnic La Fe (Valencia, Spain); the project received the approval of the hospital's medical ethics committee (registration number: 2020-268-1). The main objective was to study by real-time PCR (RT-PCR) the presence of viral RNA from SARS-CoV-2 as well as the expression of ACE2 receptor on the endometrial tissue.

Participants/materials, setting, methods: 15 women in the reproductive age (24-46 years) accepted to participate in the study and signed the informed consent. All these patients tested positive for SARS-CoV-2 by RT-PCR of nasopharyngeal swabs (1-17 days before the biopsy collection) and were hospitalized due to health complications (pneumonia) derived from COVID-19. Endometrial biopsies were taken by aspiration and preserved in RNA-later until -80°C cryopreservation in a biobank; RNA was extracted for RT-PCR for N1, N2, and ACE2 genes.

Main results and the role of chance: The 15 recruited patients represented the different phases of the menstrual cycle: proliferative (n=3) and secretory (n=10); 2 patients had amenorrhea. The viral RNA for SARS-CoV-2, measured by the detection of N1 and N2 gene targets (fragments of N gene, from the viral nucleocapsid) by RT-PCR methodology, was undetectable in all the endometrial biopsies analyzed (n=15). In all the cases the housekeeping gene RPP30 was used as positive control and to check RNA integrity. To correlate the presence or absence of SARS-CoV-2 with the organ-specific expression of ACE2 (angiotensin-converting enzyme 2), the main postulated entry receptor of SARS-CoV-2, the endometrial RNA was also analyzed by RT-PCR for the ACE2 receptor gene. This gene was only detectable in 10 of the 15 biopsies, and the levels ranged from 28.65 to 36.19 Ct values, revealing a very low expression of ACE2 in the tissue. Moreover, ACE2 results did not report any correlation with the phase of the menstrual cycle.

Limitations, reasons for caution: These results imply endometrium is safe from SARS-CoV-2 infection, at least in the short term. All the endometrial samples were taken at maximum of 17 days after a positive test by RT-PCR of nasopharyngeal swabs (to note that all were hospitalized during the early stages of the disease).

Wider implications of the findings: In conclusion, the SARS-CoV-2 RNA is not present in the human endometrial tissue of positive patients. This hypothesis was reinforced by the low ACE2 receptor levels. However, an in-depth genetic analysis comparing to a negative control group could elucidate a systemic affectation of the endometrium, despite the negative RT-PCR results.

Trial registration number: not applicable

O-143 Characterization of vaginal and endometrial microbiome in patients with chronic endometritis (CE).

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Study question: Could vaginal and endometrial microbiome by sequencing 16S rRNA be comparable to classic diagnostic methods or immunohistochemistry CD138 for diagnosis of chronic endometritis?

Summary answer: A characteristic endometrial and vaginal microbiome is present in patients with chronic endometritis. An abnormal vaginal microbiome correlates with the presence of chronic endometritis.

What is known already: Chronic endometritis is a disease characterized by persistent inflammation of the endometrial lining. Currently, histopathological evaluation by immunohistochemistry CD138 marker is most common diagnostic method for CE. Microbiome analysis based on subunit 16S rRNA sequencing is a fast tool that can enable the identification of pathogenic microorganisms associated with CE. The main bacteria at vaginal and endometrial level belong to genus *Lactobacillus*, producers of lactic acid that allows maintaining acidic pH of vagina and acts as barrier against pathogens. Investigations on the effect of an abnormal endometrial and vaginal microbiome could improve assisted reproductive technologies.

Study design, size, duration: This is a observational pilot study (60 patients and 120 samples). The study population consists of patients attending to our fertility clinic for frozen euploid embryo transfer (FET) from May 2017 to May 2019. Preimplantation Genetic Testing of aneuploidy (PGT-A) was performed at blastocyst stage using Veriseq (Illumina). The inclusion criteria to be meet by patients were: age between 18 and 50 years, own or donated oocytes and use of ICSI.

Participants/materials, setting, methods: Cohort study with sixty patients undergoing assisted reproductive treatment (TRA) with their own or donated gametes and PGT-A Vaginal and endometrial samples were taken in the cycle prior to embryo transfer. The vaginal and endometrial microbiome was analyzed by mass sequencing of the V3V4 region of 16S rRNA. Bioinformatics analysis was performed using QIIME2 and MicrobiomeAnalyst packages. Alpha, beta diversity and taxonomic characterization were compared with positive and negative CD138 groups for chronic endometritis (CE).

Main results and the role of chance: Different bacterial communities were detected when vaginal and endometrial samples were analyzed in patients with and without endometritis diagnosed with CD138 immunohistochemistry. In patients with endometritis, a higher alpha diversity index tendency was found in vaginal samples (p=0.15 for the Shannon index) and significant differences in endometrial samples (p=0.01 for the Shannon index). In the beta diversity analysis, no significant differences were observed between the groups established as per the diagnosis of endometritis. Vaginal and endometrial samples from women with endometritis showed a microbiome pattern not dominated by *Lactobacillus* spp. Relative abundance analysis identified the genera *Ralstonia* and *Gardnerella* in endometrial sample, and the genera *Streptoccoccus* and *Ureaplasma* in vaginal sample of patients diagnosed with CD138 for endometritis. Comparing endometrial and vaginal samples CD138 positive diagnosed for endometritis, alpha diversity (p=0.06 for the Shannon index and p=0.08 for the Simpson index) and beta diversity (p<0.001) showed significant differences. Relative abundance identified the genera *Lactobacillus* (p=3.76E-4), *Ralstonia* (p=8.19E-4), *Delftia* (p=0.004) and *Anaerobacillus* (p=0.004) in these sample groups.

Limitations, reasons for caution: The main limitation of this study is the small sample size. Larger studies including a higher number of samples are needed to confirm the different microbiome pattern observed at the vaginal and endometrial levels in correlation with chronic endometritis. The microbiome pattern has not been analyzed after treatment of CE.

Wider implications of the findings: Our findings suggest the existence of a characteristic vaginal and endometrial microbiota in patients with chronic endometritis. Different genera and species were identified in patients with and without endometritis depending on whether the sample was endometrial or vaginal. An abnormal vaginal microbiome appears to be strongly correlated with chronic endometritis.

Trial registration number: Not Applicable

O-144 Endometrial production of 17β-estradiol in relation to pregnancy outcome in women undergoing IVF/ICSI

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Study question: Does the endometrial synthesis and inactivation of 17β-estradiol differ between receptive and non-receptive endometrium in women undergoing IVF/ICSI?

Summary answer: The synthesis and inactivation of 17β-estradiol is similar in the endometrium of women who did and did not achieve a clinical pregnancy through IVF/ICSI.

What is known already: Implantation failure of high-quality embryos is a main concern in IVF/ICSI treatment. Blood sex-steroid concentrations do not reflect their corresponding concentrations in endometrial tissue. This is in line with the concept that blood steroids (and precursors) are locally converted to bioactive metabolites and vice versa, by expressing steroid-metabolising enzymes, such as 17β-hydroxy steroid dehydrogenase (17β-HSD). Studies indicate that alterations in intracrinology might modulate endometrial receptivity. We hypothesize that the local 17β-HSD activity during the window of implantation (WOI) differs between pregnant and non-pregnant IVF/ICSI patients.

Study design, size, duration: Case-control study of 40 patients that were recruited in the SCRaTCH study (NL5193/NTR5342), a randomised trial exploring whether 'endometrial scratching' in patients with a previous IVF/ICSI cycle

failure affects pregnancy outcome in a subsequent IVF/ICSI cycle. For the present investigation, 20 endometrial biopsies from women who achieved clinical pregnancy after fresh embryo transfer (ET) were compared with 20 endometrial biopsies of women that did not conceive after fresh ET.

Participants/materials, setting, methods: Endometrial biopsies and serum were obtained at LH+5-8 days (urinary test) in a natural cycle, prior to the fresh ET cycle. Cases (negative pregnancy test, n=20) and controls (clinically pregnant, n=20) were matched for primary vs. secondary infertility, embryo quality and age. Reduction of estrone to 17β-estradiol (synthesizing 17β-HSDs) and oxidation of 17β-estradiol to estrone (inactivating 17β-HSDs) were determined by high-performance liquid chromatography (HPLC). Results were compared with the Wilcoxon-Mann-Whitney Rank Sum Test.

Main results and the role of chance: Activity of 17β-HSDs responsible for the reduction of estrone to 17β-estradiol (mainly 17β-HSD type 1) was detected in all samples and ranged from 55 to 1864 pmol 17β-estradiol formed/mg protein/24 h. The values obtained from pregnant women (median: 1054) were not significantly different to those obtained from non-pregnant women (median: 997), p=0.97. The activity of enzymes responsible for the oxidation of 17β-estradiol (mainly 17β-HSD type 2) into the less active estrone ranged from 32 to 1731 estrone formed/mg protein/24 h. The values obtained from pregnant women (median: 737) were not significantly different to those obtained from non-pregnant women (median: 624), p=0.90. The ratio of 17β-HSD type 1:17β-HSD type 2 had a median of 1.63 in the pregnant woman compared to 1.95 in the group of non-pregnant woman (p=0.57).

Limitations, reasons for caution: The study is pilot in nature and study population is small. Primary and secondary infertility patients were analysed together. A chance phenomenon could have occurred as included women were included after their first IVF/ICSI cycle, hence not every included study person met the criteria for repeated implantation failure (RIF).

Wider implications of the findings: 17β-estradiol metabolism takes place during the WOI, controlling the final 17β-estradiol level. Although the present investigation did not show differences between pregnant and non-pregnant women, it remains important to explore if estrogen balance deviations, e.g. in other cycle phases plays a role in clinical conditions such as primary infertility/RIF.

Trial registration number: NL5193/NTR5342

O-145 Green Tea catechins EGCG and pro-drug of EGCG (Pro-EGCG) inhibit endometriosis through targeting molecules regulating macrophages and B cells

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Study question: What are the therapeutic targets and mechanisms of green tea EGCG and Pro-EGCG in treating endometriosis?

Summary answer: EGCG and Pro-EGCG have unique molecular targets to regulate interactions of B cells, macrophages and endometriotic cells and limit the growth and development of endometriosis.

What is known already: Current treatments of endometriosis are mainly hormonal suppression and surgical ablation or removal. Our previous studies showed EGCG significantly inhibits development of experimental endometriosis in mice. Pro-EGCG is more effective than EGCG in term of anti-endometriosis, anti-angiogenesis and anti-oxidation (Wang, et. al., 2013; Xu, et al., 2011). Dysfunctional immunological activities of macrophages and B cells were found in women with endometriosis. The molecular targets, underlying mechanism and differential therapeutic efficacy of EGCG and Pro-EGCG, as well as their anti-inflammatory activities are still not known.

Study design, size, duration: Multiplexed Proteome Integral Stability Alteration (PISA) assay (Gaetani et al.,2019), followed by MS/MS was applied to identify the molecular targets of EGCG and Pro-EGCG in endometriotic cells. Pharmacological studies of EGCG and Pro-EGCG on endometriotic cell line and endometriosis models in mice were performed to characterise their anti-endometriosis and anti-inflammatory effects. Gene silencing and over-expression experiments were conducted to confirm the immunoregulatory mechanisms.

Participants/materials, setting, methods: Endometriotic (Hs832(C)T) cell lines in culture and lysate were treated for chemical proteomics analysis. SiRNA and overexpression vectors were transfected to the cells *in vitro* and lesions *in vivo*. Hs832(C).T, monocytic cells (THP-1) and control B cell (Raji null) lines were used for co-culture assays to study the interaction between endometriotic and immune cells *in vitro*. Endometriosis mice model was established for immunostaining and microarray analysis of lesions to characterise the molecular pathways *in vivo*.

Main results and the role of chance: MTDH and PXK were the strongest and most differential targets of EGCG and Pro-EGCG in both cells lysate and cell culture of Hs832(C).T, respectively. Gene silencing and overexpression of the protein targets *in vitro* and *in vivo* significantly altered expressions of downstream proteins, including BLK and EGF after PXK, and MYC and AKT after MTDH, as well as endometriosis-related genes such as VEGFC and MMP9. Co-culture assays of Hs832(C).T with Raji null or THP-1 induced macrophages showed that expressions of PXK, MTDH, downstream targets, and immune-related genes were significantly increased after incubation of recombinant proteins, but were significantly decreased after EGCG and Pro-EGCG treatment. M1 and M2 macrophages, as well as B cells were significantly reduced after the treatments *in vitro* and *in vivo*. Double immunofluorescent staining of lesions showed that CD68, CD163 or CD20 co-expressed with MTDH, PXK and downstream targets, and numbers of the co-expressed cells were significantly reduced after treatments *in vivo*. Microarray experiment further identified the upstream and downstream genes of MTDH or PXK contributing to the growth and development of endometriosis.

Limitations, reasons for caution: Results of this pharmacological and mechanistic study require clinical samples to validate the anti-endometriosis effects of EGCG and Pro-EGCG. Effects of other potential pharmaceuticals targeting the macrophages and B cells on endometriosis are needed.

Wider implications of the findings: The findings provide pharmacological and mechanistic data for future development of EGCG and Pro-EGCG as new treatment for endometriosis. This study shows that macrophage and B cell could be potential therapeutic targets for treatment of endometriosis, which opens up new horizon for the novel immunotherapy for endometriosis.

Trial registration number: NA

O-146 Assessment of focal and diffuse adenomyosis lesions before and after pregnancy on magnetic resonance imaging : a cohort of 139 patients.

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Study question: How to assess the different adenomyosis phenotype before and after pregnancy on magnetic resonance imaging according to stringent validated criteria ?

Summary answer: Diffuse adenomyosis increases significantly after pregnancy while the rate of focal adenomyosis and the mean volume of focal adenomyosis lesions decrease significantly after pregnancy.

What is known already: Adenomyosis and endometriosis are benign hormone-dependent disorders associated with pelvic pain, dysmenorrhea and/or infertility. The natural course of adenomyosis and endometriosis is still unclear, particularly during pregnancy. Pregnancy is considered to have a positive impact on endometriosis. Several studies regarding the impact of adenomyosis on pregnancy are available. Adenomyosis can cause fertility disorders, miscarriage, preterm birth. However, available data evaluating the effect of pregnancy on adenomyosis are lacking.

Study design, size, duration: Between January 1st 2010 and September 30th 2020, 139 patients were followed in our referral care center (Gynecology department of Port-Royal Hospital, Paris) for symptomatic adenomyosis and or endometriosis. For each of them, a magnetic resonance imaging were performed before and after pregnancy. The data based on magnetic resonance imaging, pre- and post-pregnancy, were analyzed in a single retrospective study.

Participants/materials, setting, methods: Patients had to be over 18 years old, to be pregnant and to be followed for symptomatic adenomyosis

or endometriosis without any previous surgery. Each pelvic magnetic resonance imaging were performed by a single experienced radiologist. The protocol was identical on a 1.5 T magnetic resonance imaging machine based on validated criteria. The rate of diffuse and focal adenomyosis, the volume of focal adenomyosis lesions and the thickness of maximal junctional zone were reported.

Main results and the role of chance: The mean age of patients was 34.6 ± 3.4 years old, 83 (59.7%) of patients underwent assisted reproductive technology to be pregnant. The mean time interval between the MRI and the delivery was 55.2 months and the mean time interval between the delivery and the MRI was 32.2 months. Before pregnancy, there was 96 (69.1%) patients with adenomyosis, all phenotype combined versus 111 (79.9%) after pregnancy (p=0.04) on magnetic resonance imaging. The rate of diffuse adenomyosis increased significantly on magnetic resonance imaging after pregnancy compared to before pregnancy (n=22 (15.8%) vs n=41 (29.5%), p =0.01). The thickness of junctional zone maximal was significantly higher after pregnancy (8.0 mm ± 5.1 vs 12.0 mm ± 4.8, p<0.01). The rate of focal adenomyosis (n=55 (39.6) vs n=34 (24.5), p=0.01) as well as the volume of focal adenomyosis lesions (6.7 mm^3 ± 2.5 vs 6.4 mm^3 ± 2.3, p<0.01) decreased significantly after pregnancy on magnetic resonance imaging.

Limitations, reasons for caution: This single-center study was conducted in a referral center whom patients presented more severe forms of adenomyosis, which could have affected the external validity of this study. The mean time interval between delivery and MRI was 32.2 month which implies a short follow up period to observe long term outcomes.

Wider implications of the findings: The hypothesis that a specific hormonal environment during pregnancy may imply a positively impact of the evolution of focal adenomyosis is raised by this study. The evolution of focal adenomyosis after pregnancy is similar to the evolution of endometriosis lesions volume that support shared etiopathogenic mechanisms between the two entities.

Trial registration number: 'not applicable'

SELECTED ORAL COMMUNICATIONS

SESSION 47: BIOMARKERS OF MALE FERTILITY POTENTIAL

30 June 2021 Stream 2 10:00 - 11:30

O-147 Differential seminal metabolomic signature is related to sperm quality

N.M. Molina[1,2], A. Sola-Leyva[1,2], I. Pérez-Prieto[1], E. Vargas[3], M. Molina[4], A. Yoldi[4], A. Vaquero[4], P. Navas[4], A. Clavero-Gilabert[5], M.C. Gonzalvo-López[5], N. Morales[5], J.P. Ramírez[4], J.A. Castilla[2,4,5], C.M. Aguilera[2,6,7,8], S. Altmäe[1,2,9]

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[8]Instituto de Salud Carlos III, CIBEROBN CIBER Physiopathology of Obesity and Nutrition, Madrid, Spain ;
[9]Competence Centre on Health Technologies, University of Tartu, Tartu, Estonia

Study question: What is the entire metabolomic profile of human semen and does the metabolic composition differ between men with good-quality and low-quality semen?

Summary answer: Human semen contains ~700 different metabolites, and the metabolomic signature differs between normozoospermic men and men with altered seminal parameters.

What is known already: Semen contains a wide diversity of metabolites as has been identified in single and targeted metabolite studies. The full composition of metabolites in human semen, however, is not known. The knowledge of the complete metabolic signature in semen and whether there are differences between metabolic composition and seminal quality could enhance our knowledge of possible factors involved in reduced sperm quality and male infertility.

Study design, size, duration: Case-control study, where a total of 100 men (age= 29.73±8.9 years) from March 2019 to March 2020 participated. The study was approved by the Ethics Committee of Investigación Biomédica de Andalucia.

Participants/materials, setting, methods: Semen samples from 69 normozoospermic and 31 oligozoospermic men were collected at the University Hospital and sperm biobank (Ceifer Biobank - NextClinics). The complete metabolome from unprocessed seminal samples was analysed by Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS). Raw data were extracted, peak-identified and quality control processed using Metabolon's hardware and software (metabolon.com). Multiple regression models controlling for age and sample collection centres were applied using R software.

Main results and the role of chance: In total, 695 different metabolites were detected in the seminal samples, where docosahexaenoate (DHA; 22:6n3, PubChem ID 445580), choline phosphate (1014), dihomo-linolenate (20:3n3 or n6, 5280581), docosapentaenoate (n6 DPA; 22:5n6, 6441454), adenosine 3',5'-cyclic monophosphate (cAMP, 6076) and N-acetylalliin (122164824) metabolites were the most prevalent. The seminal metabolomic profiles differed significantly between men with normal and low sperm parameters. The most abundant metabolites in normozoospermic men belonged to Lipid Super-Pathway, while Nucleotide Super-Pathway was predominant in semen samples with low quality (p<0.05). More specifically, the leading Sub-Pathway in normozoospermic men was Long Chain Polyunsaturated Fatty Acid (n3 and n6), whereas Purine and Pyrimidine Metabolism Sub-Pathway prevailed in low-quality semen samples, where DHA and cAMP dominated in men with normal and low seminal quality parameters, respectively (p<0.05 in all comparisons).

Limitations, reasons for caution: This is the first study presenting the entire metabolome signature of unprocessed human semen, and these preliminary results need to be confirmed in a bigger sample size.

Wider implications of the findings: Semen analyses applied in clinics do not evaluate the functional status of sperm, leaving the infertility causes due to male factor frequently unknown. Our study results could help to understand the molecular background of reduced seminal quality and male infertility and lead to identification of molecular biomarkers of functional sperm.

Trial registration number: not applicable

O-148 Sperm Aminopeptidase N as a predictive biomarker of blastocyst development and embryo viability.

N. Subiran[1,2,3], I. Urizar-Arenaza[1,2], I. Muñoa-Hoyos[1,2], J. Irazusta[1], Z. Larreategui[4], N. Garrido[5], M. Gianzo[1]

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Study question: To evaluate human sperm APN as a prognostic factor for determining high-quality embryos.

Summary answer: The human sperm APN has the potential to become new molecular prognostic biomarker for having high-quality and viable embryos.

What is known already: Prognosis and diagnosis of male fertility is one of the major concerns in reproductive medicine. Approximately 30%-40% of men with otherwise normal fertility parameters are still unable to achieve pregnancy. The predictive clinical value of a semen analysis to identify fertile or infertile males is limited; therefore, new sperm diagnostic or prognostic methodologies are urgently required. Sperm Aminopeptidase N (APN) may be a relevant molecular marker due to its high concentration in sperm cells and its role in sperm physiology, such as motility, acrosome reaction, and embryo development.

Study design, size, duration: A prospective study that involves a total of 81 couples and 611 embryos who underwent oocyte-donation cycles at the Clínica IVI Bilbao (Spain) between September 2014 and July 2015.

Participants/materials, setting, methods: This study was set in an assisted reproduction unit and in an academic research laboratory. All semen samples were examined and classified following WHO guidelines. Spermatozoa were isolated from semen on discontinuous colloidal silica gradient (45%-90%) technique. Embryo quality and development were determined according to the Spanish Association of Reproduction Biology Studies (ASEBIR) criteria. Flow cytometry analyses of quantitative and semi-quantitative sperm human APN levels.
Main results and the role of chance: The obtained results proved that the most evolved and viable blastocysts were associated with low sperm APN levels. Expanding, expanded, hatching/hatched and viable blastocysts come from semen samples which showed lower APN levels than early blastocysts, blocked and non viable blastocyst. The cumulative probability of having more evolved blastyocysts increased 1.38-fold at day 5 and 1.98-fold at day 6 of embryo development as well as the likelihood of having viable embryo increased 1.48-fold when semen samples with low APN levels are used during the ICSI technique.
Limitations, reasons for caution: Data obtained from a single Fertility Clinic. A multi-centrum study will be required.
Wider implications of the findings: The human sperm APN has the potential to become new molecular prognostic biomarker for having high-quality embryos that could help to diagnose male infertility, especially when seminal parameters are close to the threshold values.
Trial registration number: Not applicable

O-149 Evaluation of structural problems in the application of strict criteria for sperm morphology assessment

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[3]Ziekenhuis Sint Jansdal, Clinical Chemistry, Harderwijk, The Netherlands

Study question: Which criteria, described by WHO 2010, cause most problems during sperm morphology assessment and lead to outcome variation in a national external quality control program?
Summary answer: Assessment of head ovality, regularly contoured head, regularly contoured midpiece and alignment of major axis of midpiece and head lead to the most variation.
What is known already: Morphology assessment of spermatozoa is known as a rather difficult part of semen analysis. Over the past 40 years, morphological criteria became stricter and reference values changed significantly, leading to a 4% lower reference value for normal/typical morphology in 2010. This has consequences for the statistical power of the analysis. Moreover, many laboratories do not use the staining method as advised by the WHO and are getting stricter and stricter in the application of the criteria. Improvement of the assay is therefore necessary. In this study, as a first step, variation in the use of the strict criteria is evaluated.
Study design, size, duration: Data from the Dutch external quality control (EQC) program were evaluated in this retrospective study over the period 2015 – 2020. The program consists of four rounds per year and includes the assessment of three photos of Papanicolaou stained spermatozoa. These spermatozoa were dichotomously judged (normal/abnormal) on 14 morphological criteria (WHO manual, 2010). Consensus results of three experts served as reference. In total, variation over results of 72 photos (1008 values) was analysed.
Participants/materials, setting, methods: Participants were staff members from Dutch laboratories (1 member per lab per round) that perform semen analysis. The outcomes of the participants were tested for variation per criterion, both over the entire 6-year period and for trends during this period. To gain insight in the influence of "time", three photos were provided three times (in 2015/2018/2020) and six photos were provided twice (in 2016/2018 and 2018/2020). Setup was blinded to both participants and experts.
Main results and the role of chance: In the period 2015 – 2020, 88 – 103 laboratories participated in the EQC program. Of these laboratories, 40 – 60 took part in the photo evaluation. Variation per criterion was expressed in categories green, orange and red, with resp. >90%, 60-90% and <60% agreement between the participants. Overall, variation was in 57% in category green, 37% orange and 6% red. Head ovality, regularity of head contours, regularity of midpiece contours and alignment of the major axis of midpiece and head lead to the most variation. For these criteria, resp. 14, 17, 10 and 17% were in the category red and resp. 50, 47, 71 and 64% in category orange. Lowest variation was found for acrosomal vacuoles, excessive residual cytoplasm, tail thickness and tail length with resp. 76, 77, 94, 85% in category green. Trend analysis lead to similar conclusions: most criteria show a slightly positive trend, but head ovality and regularity of head and midpiece show a stable or declining trend. Three photos were used in three rounds and six photos in two rounds. In 26 (8.8%) cases, shifts towards higher (5) or lower (21) variation were found. Experts changed their opinion in 3 (1%) cases.
Limitations, reasons for caution: Results are dependent on the morphology of the spermatozoa (magnitude of abnormalities) and of the photo quality.
Wider implications of the findings: The definitions of the criteria need to be better explained and trained, especially for ovality of the head, regularity of the midpiece and overlap of the longitudinal axes of midpiece and head. Moreover, explanation of the criteria in the light of physiology will probably lead to better evaluations by participants.
Trial registration number: Not Applicable

O-150 Predicting the sperm retrieval of microsurgical testicular sperm extraction (mTESE) in infertile men with azoospermia on the day of TESE-ICSI

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Study question: Is the sperm retrieval rate of a small, pre-processed sample (PPS) of each TESE-biopsy representative for the sperm outcome on the day of ICSI?
Summary answer: The analysis of a PPS reliably reflects the probability of finding comparable numbers of sperm at time of TESE-ICSI.
What is known already: Azoospermia is defined as a condition where no spermatozoa are found in the ejaculate and is diagnosed in up to 15% among infertile men and in 11% of all patients attending our centre. The combination of testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) has become the standard treatment of azoospermic patients. However, no validated standard procedure has been identified to predict the exact sperm outcome of the cryopreserved TESE samples prior to TESE-ICSI so far. For optimal management of TESE-biopsies and the respective ICSI treatment, we developed a stepwise approach for the analysis of tissue samples.
Study design, size, duration: We retrospectively analysed the outcome of 872 microsurgically retrieved testicular biopsies of 198 patients of legal age who had a TESE-ICSI at our department between 2009 and 2019. From all 872 mTESE biopsies the number of sperm extracted from a small, pre-processed sample (PPS) before freezing procedure were known. The PPS was then compared to the number of sperm retrieved from the corresponding thawed specimen on the day of TESE-ICSI.
Participants/materials, setting, methods: During micro-TESE eight samples per testis are retrieved, then 1/10 of each biopsy is removed, digested with collagenase and screened for spermatozoa (pre-processed sample, PPS). If less than 100 spermatozoa are detected the absolute sperm number is recorded, otherwise the result is displayed as the maximum value of 100 sperm. On the day of ICSI, one or more TESE biopsies are thawed and processed for TESE-ICSI; the absolute sperm number is counted again.
Main results and the role of chance: Comparing the sperm yield of 872 TESE samples at time of ICSI to its respective PPS showed a similar sperm outcome with a minor deviation of ±5 spermatozoa in 73.6% of all biopsies. However, 12.9% of the specimen had less and 13.4% had more spermatozoa. A negative sperm retrieval in the initial PPS was confirmed in 93.1% (268/288). PPS with 1-4 spermatozoa had a 27.2% (43/158) risk of complete absence of sperm on the day of ICSI, yet sperm detection (≥1 sperm) was positive in 72.8% (115/158) of the biopsies. With initially ≥5 spermatozoa present in the PPS, only 0.9% (4/426) of the biopsies had no sperm on the day of ICSI, vice versa 99.1% (422/426) were spermatozoa positive. A significant (p=0.01) and strong (rs= 0.926) correlation of the sperm retrieval rates of the PPS and the ICSI sample was found meaning that the PPS reflects very well the sperm retrieval rate of the cryopreserved mTESE biopsy thawed at time of TESE-ICSI. However, if ≤4 sperm are found in the PPS, there is a relevant risk for a negative sperm

retrieval on the day of ICSI and the couple should be carefully advised before start of treatment.
Limitations, reasons for caution: This analysis focussed on sperm prediction in cases of severe male factor infertility and therefore the sperm yield on the day of ICSI was chosen as primary outcome. The reproductive competence of the retrieved sperm in terms of pregnancy and birth rates should be subject to further investigation.
Wider implications of the findings: Treatment options for azoospermic patients are mostly related to the ability to find sperm on the day of ICSI. However, validated standards for sperm processing are missing. Therefore, a PPS seems to be a good option for prediction of sperm retrieval and improves counselling of the patients prior to TESE-ICSI.
Trial registration number: not applicable

O-151 Detailed characterization of infertile men with idiopathic versus unexplained infertility: a single-center experience

L. Boeri[1], L. Candela[1], E. Pozzi[1], F. Belladelli[1], P. Capogrosso[2], W. Cazzaniga[1], A. Costa[1], G. Fallara[1], N. Schifano[1], D. Cignoli[1], E. Ventimiglia[1], M. Alfano[1], C. Abbate[1], F. Montorsi[1], A. Salonia[1]

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Study question: We aimed to investigate the rate of and the clinical characteristics of men with idiopathic versus unexplained infertility from a cohort of white-European men.
Summary answer: Approximately 20% and 5% of men evaluated for primary couple's infertility depicted characteristics suggestive for idiopathic and unexplained infertility, respectively.
What is known already: Male factor infertility (MFI) can be associated with clinical, hormonal and genetic diseases, but MFI is idiopathic in almost 30% of cases.
Study design, size, duration: Data from 3098 infertile men (according to WHO definition) consecutively evaluated between 2003-2020 at a single academic centre were analysed and compared with those of 103 fertile controls. Health-significant comorbidities were scored with the Charlson Comorbidity Index (CCI). Idiopathic infertility was defined for pathological semen analysis but normal physical examination and endocrine, genetic and biochemical laboratory testing. Unexplained infertility is defined as infertility of unknown origin with normal sperm parameters.
Participants/materials, setting, methods: Testicular volume (TV) was assessed with a Prader's orchidometer. Serum hormones and sperm DNA fragmentation index (SDF) were measured in every patient. Vitamin D3 (VitD) deficiency was considered for vitD levels <20 ng/mL. Semen analyses were based on the 2010 WHO reference criteria. Health-significant comorbidities were scored with the Charlson Comorbidity Index (CCI).
Main results and the role of chance: Overall, 570 (18.5%) and 154 (5.0%) patients depicted criteria suggestive for either idiopathic or unexplained primary infertility, respectively. Groups were similar in terms of age, BMI, CCI, recreational habits, circulating serum hormones and SDF. Testicular volume was lower in men with idiopathic vs. unexplained infertility [median (IQR) 20 (15-25) vs. 20 (17-25); p<0.001]; more idiopathic than unexplained infertile men depicted TV <15ml (23.4% vs. 12%; p<0.01). Similarly, vitD levels were lower [22 (17-28) vs. 27 (21-42) ng/mL; p<0.001] in idiopathic vs. unexplained infertile men, with a higher rate of pathologic VitD levels in the same group (42.1% vs. 10%; p=0.04). When compared to fertile controls, groups were similar in terms of age, BMI, CCI and serum hormones values. TV was larger in fertile controls than idiopathic and unexplained infertile men (all p<0.01). At multivariable logistic regression analysis only vitD deficiency (OR 8.1, p=0.03) was found to be associated with idiopathic infertility after accounting for age, BMI, testosterone values and TV.
Limitations, reasons for caution: The small number of fertile controls may raise the possibility of biases.
Wider implications of the findings: Idiopathic and unexplained infertility were identified in approximately 20% and 5% of men evaluated, respectively. Idiopathic infertile men showed lower TV and lower vitD values compared to men with unexplained infertility. Future studies are needed to develop a more tailored management to these difficult MFI cases.
Trial registration number: .

O-152 Investigation of the relationship of sperm motility and Kisspeptin in subfertile men

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Study question: Does kisspeptin administration affect the motility parameters in sperm samples of subfertile cases?
Summary answer: Kisspeptin administration significantly increased gene expression levels related with sperm motility as well as intracellular calcium concentrations.
What is known already: Sperm motility problems are among the most important causes of male infertility. In recent years, a peptide named kisspeptin has been discovered that may have effects on sperm motility. Kisspeptin is known to trigger calcium release in hypothalamic neurons. In addition, kisspeptin administration increased sperm progressive motility in studies conducted on normozoospermic individuals. Furthermore, it is suggested that kisspeptin protein in seminal plasma is positively associated with semen quality. However, there is no evidence that how kisspeptin can affect sperm in men with infertility problems.
Study design, size, duration: This basic research study was an in vitro experimental approach involving the use of semen samples from an infertil cases between September to December in 2020. 40 men were included in both control and experimental groups.
Participants/materials, setting, methods: All analyses were performed on semen samples from 10 normozoospermic (NZ), 10 asthenozoospermic (AZ), 10 oligoasthenozoospermic (OAZ) and 10 oligoastenoteratozoospermic (OATZ) men, aging between (21-40) years. Basal serum and seminal kisspeptin levels were analyzed by ELISA. Sperm were divided into two groups. Kisspeptin-13 administered *in vitro*. *KISS1, KISS1R, CATSPER1, AKAP4* gene expressions analyzed by qRT-PCR using $2^{-\Delta\Delta Ct}$ algorithm. Intracellular calcium concentration was determined with floresence spectroflurometer and laser scanning confocal microscope.
Main results and the role of chance: The serum kisspeptin level of NZ was significantly higher than other groups (p<0.05). The semen kisspeptin level was significantly higher than OAZ and OATZ (p<0.05), but not in NZ (p>0.05). Also, *KISS1* gene expression was higher in AZ compared to other groups (p<0.05). Biochemical and gene expression analysis of kisspeptin were consistent with each other. There was a significant increase in the expression of *CATSPER1* gene in AZ compared to other groups (p<0.05). Also, *AKAP4* gene expression was significantly higher in OATZ compared to other groups (p<0.05). No significant difference was documented for the expression of *KISS1R* (p>0.05). Intracellular calcium was significantly increased in AZ and NZ after kisspeptin administration. The intracellular calcium increase is consistent with increased *CATSPER1* gene expression levels in AZ. Kisspeptin administration may have a significant effect on sperm motility parameters.
Limitations, reasons for caution: The biochemical and gene expression levels of *KISS1* were consistent. However, gene expression was explored at the mRNA level for *CATSPER1* and *AKAP4*. The protein expression analyses of these genes may confirm the results. Also, using kisspeptin antagonists may strength the results of intracellular calcium analysis.
Wider implications of the findings: Kisspeptin treatment for individuals diagnosed with asthenozoospermia may have therapeutic results. KISS1 quantitation may be a determining factor for the subfertility in routine semen analysis.
Trial registration number: OMU KAEK 2019/462

SELECTED ORAL COMMUNICATIONS
SESSION 48: DETERMINANTS OF EMBRYO QUALITY
30 June 2021 Stream 3 10:00 - 11:30

O-153 Is paternal age associated with timing, stage, morphology, and implantation of the competent blastocyst: a multicenter cohort study

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Study question: Is age of men undergoing assisted reproductive technology associated with timing, stage, morphology, and implantation of the competent blastocyst?
Summary answer: Advanced paternal age was associated with reduced development speed and level of hCG in the competent blastocysts in COS treatments.
What is known already: We have shown that for every one-year increase in women's age there is a 5% reduced probability that the competent blastocyst is in an advanced development stage. Likewise, we have shown that the initial hCG rise is associated with women's age with the youngest women having the lowest hCG level. It is unknown whether the age of men undergoing ART treatment is associated with the timing (day 5 or 6), the development stage, morphology, and the early implantation of the competent blastocyst.
Study design, size, duration: This is a multicenter historical cohort study based on data from 16 private and university-based public fertility clinics where 7246 men and women, between 2014 and 2018, underwent controlled ovarian stimulation (COS) or Frozen-thawed Embryo Transfer (FET) with a single blastocyst transfer resulting in a singleton pregnancy. These data were linked to the Danish Medical Birth Registry, resulting in inclusion of 4842 men with a partner giving birth.
Participants/materials, setting, methods: Exposure (age) and outcome data (blastocyst timing (day 5 or 6), development stage (3-6), inner cell mass (ICM)(A,B,C), trophectoderm (TE)(A,B,C) and hCG were collected from the database, Danish Medical Data Center. All COS cycles (IVF and ICSI) and FET cycles (natural and substituted), were included. Exclusion criteria were cycles with pre-implantation genetic testing, donated oocytes and semen. The analyses were adjusted for female age, female smoking, female BMI, diagnosis and clinic.
Main results and the role of chance: The adjusted association between paternal age and transfer day in COS treatments showed that for every increase of one year, men had a 6% increased probability that the competent blastocyst was transferred on day 6 compared to day 5 (OR 1.06, 95% CI (1.00;1.13). The mean difference in hCG values when comparing paternal age group 30-34, 35-39 and 40-45 with the age group 25-29 in those receiving COS treatment, all showed significantly lower adjusted values for older men. In FET treatments, none of the investigated associations reached statistical significance.
Limitations, reasons for caution: The blastocyst morphology was subjectively assessed, and information bias may have influenced the results. However, adjusting for clinic takes the potential influence of variation in embryo scoring between clinics into consideration.
Wider implications of the findings: We hypothesize that the later transfer (day 6) in female partners of older men is likely to be due to longer time spent by the oocyte to repair fragmented DNA of the sperm cells, which should be a focus of future research in men.
Trial registration number: "not applicable"

O-154 First mitotic spindle formation led by sperm centrosome-dependent microtubule organising centres may cause high incidence of zygotic division errors in humans

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Study question: Why do multinucleated blastomeres appear at high frequency in two-cell-stage embryos in humans?
Summary answer: Failure in microtubule assembly during the first mitotic spindle body formation by sperm centrosome-dependent microtubule organising centres (MTOCs) may lead to chromosomal instability.
What is known already: Unlike that in mice, multinucleated blastomeres appear at high frequency in two-cell-stage embryos in humans. However, the underlying mechanism remains elusive. In mice, multiple acentriolar MTOCs appear around the male and female pronuclei after pronuclear disappearance and contribute to dual-spindle formation, engulfing each parental chromosome. This spindle formation may ensure an error-free division, keeping the chromosomes stable during the first cleavage, as observed in mice, but it is unclear whether a similar mechanism exists in humans.
Study design, size, duration: To examine how sperm centrosomes contribute to MTOC formation in humans, two types of 3PN zygotes derived fromeither conventional *in vitro* fertilization (c-IVF, n = 30) or intracytoplasmic sperm injection (ICSI, n = 10) were used. The zygotes were collected from October 2018 to January 2020. MTOC and mitotic spindle formation at consecutive stages of development during the first cleavage were analysed under static and dynamic conditions using immunofluorescence assay and fluorescent live-cell imaging.
Participants/materials, setting, methods: Under ethics approval, 3PN zygotes were donated by infertile couples undergoing c-IVF or ICSI cycles at the Yamashita Shonan Yume Clinic in Japan. All participants provided informed consent. Immunofluorescence assay was performed using antibodies against -tubulin, pericentrin, and H3K9me3 after fixation with MTSB-XF solution. Fluorescent live-cell imaging was performed using TagGFP2-H2B mRNA (chromosome marker) and FusionRed-MAP4 mRNA (microtubule marker).
Main results and the role of chance: Immunofluorescence revealed that while 3PN zygotes derived from c-IVF showed four pericentrin dots, those derived from ICSI exhibited two pericentrin dots. In pro-metaphase, an independent group of chromosomes derived from each pronucleus and MTOCs were formed by the sperm centrosome at the core. Microtubules from each MTOC extended toward the chromosomes in the early metaphase; a quadrupolar spindle was formed in the c-IVF-derived zygotes, and a bipolar spindle was formed in the ICSI-derived zygotes by the MTOCs at the zygote apex after chromosome alignment. In pro-metaphase, the microtubules extended from the MTOCs to the nearest chromosome. Since microtubule assembly was found on oocyte-derived chromosomes, we hypothesised that whether a chromosome is surrounded by microtubules depends on the location of the MTOCs, irrespective of its origin. Live-cell imaging of histone H2B and MAP4 revealed that four MTOCs appeared around the three pronuclei just before the disappearance of the pronuclear membrane; microtubules then extended from the MTOCs toward the chromosomes, beginning to form a mitotic spindle as the chromosomes moved to the centre of the oocyte. Interestingly, one of the three assembled chromosome groups showed no microtubule assembly in the pro-metaphase. Similar results were obtained in all six 3PN zygotes subjected.
Limitations, reasons for caution: We demonstrated the high risk of developing bare chromosomes not surrounded by microtubules during the formation of the first mitotic spindle, using human tripronuclear zygotes. However, owing to unavailability of normal fertilized oocytes for this study because of the clinical use, we were unable to confirm this in normal zygotes.
Wider implications of the findings: Although two sperm centrosome-dependent MTOCs are expected to be formed in normal fertilized oocytes, these MTOCs are not sufficient to completely enclose physically separated female and male chromosomes with the microtubules. This explains the high frequency of zygotic division errors that lead to unstable human chromosomes.
Trial registration number: not applicable

O-155 Dietary caloric normalization or restriction as preconception care strategies: impact on oocyte developmental competence and quality in high fat/high sugar-induced obese outbred mice

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Study question: Can diet normalization or caloric restriction (CR) for two weeks be used as a preconception care intervention in obese Swiss mice to restore oocyte development and quality
Summary answer: Diet normalization or CR as short-term preconception care interventions in obese mice only partially restored oocyte quality but did improve overall developmental competence.

What is known already: Maternal metabolic disorders like obesity and metabolic syndrome may result in decreased oocyte and embryo quality, and thus reproductive failure. Overweight and obese patients are advised to lose weight before conception to increase the chance of a healthy pregnancy. However, as human studies show no univocal guidelines, more fundamental research might provide additional answers. In order to avoid interference with increased maternal age, the question remains if oocyte quality can be restored after only a short preconception care intervention (PCCI).
Study design, size, duration: Outbred mice were fed a control (CTRL) or high-fat/high-sugar (HF) diet for seven weeks. Afterwards, HF-mice were put on different PCCIs for two weeks, resulting in four treatment groups: control diet (9w; CTRL_CTRL), HF diet (9w; HF_HF), switch from HF (7w) to an *ad libitum* control diet for 2w (HF_CTRL) or to a 30% CR diet for 2w (HF_CR). Oocyte developmental competence (n=357) and quality (12-16 oocytes /treatment, scored blinded) were determined, using 6-8 mice/treatment.
Participants/materials, setting, methods: Body weight changes were recorded. *In vivo* matured oocytes were collected after superovulation and analysed for quality or *in vitro* fertilized and cultured. Oocyte quality was determined by staining for lipid content (Bodipy) and mitochondrial inner membrane potential and active mitochondria localization (JC-1). Oocyte developmental competence [cleavage (24h p.i.) and blastocyst rates (5 days p.i.)] was scored. Categorical and numerical data were analysed using binary logistic regression and ANOVA, respectively and corrected for multiple testing.
Main results and the role of chance: Compared to the CTRL group, HF diet increased body weight after 7 weeks by 24.19% ($P<0.001$). After the start of the PCCI, both HF_CTRL and HF_CR mice progressively lost weight and reached values similar to control mice after two weeks. HF_HF diet increased the intracellular lipid content in oocytes with 54.3% compared to the CTRL_CTRL group ($P<0.05$). This increased content was (partially) normalized in both preconception care intervention groups, even similar to the control levels in the HF_CTRL group. Both HF_HF and HF_CR oocytes showed a tendency to an increased ratio of active/total mitochondria when compared to the CTRL_CTRL group ($P=0.081$, $P=0.083$ respectively). In addition, active oocyte mitochondria in the HF_HF group were less pericortically distributed compared to controls. This was also the case in both preconception care intervention groups ($P<0.05$). After two weeks of PCCI, oocytes from HF_HF mice displayed lower cleavage rates than those from CTRL_CTRL mice (36.26% *vs.* 64.52%, $P<0.05$) but blastocyst rates (26.37% *vs.* 35.48%, $P>0.1$) were not different. HF_CR, but not HF_CTRL, oocytes showed higher cleavage rates (68.48%, $P<0.001$) compared with HF_HF oocytes. Moreover, both HF_CTRL (44.64%, $P<0.05$) and HF_CR (59.78%, $P<0.001$) oocytes showed improved blastocyst rates when compared to the HF_HF group (26.37%).
Limitations, reasons for caution: Although using a mouse model has several advantages, translating these results to the human setting is a limitation of this study. However, to improve this translatability, an outbred mouse model was used. Additional data will be collected to gain more information regarding the best preconception care intervention advice.
Wider implications of the findings: This research aims to provide fundamental insights in order to be able to formulate clear preconception guidelines to obese women planning for pregnancy. In addition, we aim to find the shortest possible intervention period to improve fertility.
Trial registration number: Not applicable

O-156 Fertilization rate as a novel indicator for cumulative live birth rate: multicenter retrospective cohort study of 9,394 complete IVF cycles

C. Zaca'[1], G. Scaravelli[2], P.E. Levi Setti[3], C. Livi[4], F.M. Ubaldi[5], M.T. Villani[6], E. Greco[7], M.E. Coccia[8], A. Revelli[9], G. Ricci[10], F. Fusi[11], V. Vigiliano[2], R. De Luca[2], S. Bolli[12], A. Borini[13]

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Study question: Does fertilization rate (FR) affect cumulative success rates in assisted reproduction cycles?
Summary answer: These data indicate a positive association between FR with CLBR suggesting the predictive clinical relevance of this parameter and its adoption as Key Performance Indicator(KPI).
What is known already: Numerous studies have aimed at characterizing outcome predictors. Maternal age is historically and correctly recognized as the single most important factor impacting on the clinical outcome of ART. More recently ovarian response has also gained interest in this respect. However, the quest for novel, more comprehensive predictive factors is not over; new relevant evidence is starting to emerge. FR is a noteworthy parameter because expressing a fundamental aspect of both oocyte and sperm developmental competence. In fact it has been adopted as a key performance indicator of the IVF laboratory, to assess laboratory, operator, and gamete competence.
Study design, size, duration: Reported data concern a retrospective cohort study carried out between 2015 to 2017 involving 7,968 couples undergoing 9,394 complete ICSI cycles, i.e. whose all embryos were transferred or disposed.All women aged between 18-42 years were included.We excluded from analysis: surgical sperm retrieval cases, cycles resulting in neither fresh or frozen–thawed embryo transfers,cycles in which live birth were not achieved, but with remaining cryopreserved embryos,cycles of PGT, cycle with fertilization failure and standard IVF cycles.
Participants/materials, setting, methods: The cohort was groupped according to fertilization rate intervals based on recommendations of the Vienna Consensus (<65% - Group 1; 65%-80% - Group 2; >80% - Group 3). Harnessing the large size of the original dataset, further cycle stratifications were carried out based on female age (<34, 35-38, 39-42 years) and number of oocytes retrieved (5-7, 8-10, >10 oocytes).
Main results and the role of chance: No significant difference in female age was observed between fertilization rate groups (p=0.640). CLBR was progressively higher in relation fertilization rate in Groups 1, 2 and 3 (20.1%, 34.7%, 41.3%, P<0.001, respectively). Number of recovered oocytes, embryo number per cycle, cumulative pregnancy rate followed the same trend (p<0.001). The decrease in CLBR with increasing female age was significantly correlated with fertilization rate and CLBR in all three female age groups (P<0.001). Finally, to further control for possible patient-specific confounding factors, maternal age, number of retrieved oocytes, percent of inseminated oocytes and fertilization rate were evaluated in a multivariate logistic regression analysis. From this assessment, fertilization rate emerged as a factor independently associated with cumulative live birth rate, to a degree equivalent or higher compared with the number or retrieved oocytes.
Limitations, reasons for caution: The study design is retrospective and requires further refinement to control for factors that may impact clinical outcome.
Wider implications of the findings: These data indicate a positive association of FR with CLBR, thereby suggesting that fertilization, in addition to representing an assay for gamete quality and laboratory performance,has an independent clinical significance.Irrespective of the number of retrieved oocytes and female age, we observed that, rates of FR are positively associated with CLBR.
Trial registration number: None

O-157 Micro-Computed Tomography of the adult mouse ovary: an in-silico 3D reconstruction of folliculogenesis

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Study question: Which are the spatial dynamics of follicles recruitment and growth inside the ovary?
Summary answer: 3D micro-Computed Tomography (microCT) shows a simultaneous and homogeneous distribution of follicle recruitment all-over the cortex,and subsequent growth within the same ovarian region.
What is known already: In the mouse ovary, folliculogenesis progresses from the primordial type 1 (T1) to the fully-grown T8 follicle. Most of our knowledge of the folliculogenetic process has been obtained by disaggregating the ovary into its functional units (i.e., follicles and oocytes), thus losing the complexity of the whole histo-functional context.

To date, few studies employed 3D imaging approaches to gain information on the inside 3D ovary organisation. MicroCT is the only technique that combines a high spatial resolution (down to ~1 µm) with the production of a true 3D organ reconstruction, with cubic voxels and isotropic resolution.
Study design, size, duration: Three ovaries of three different adult mice were treated with the contrast agent and then imaged with microCT. A typical experiment required a total of 35 man/h from ovaries isolation to completion of X-ray scanning, and 24 man/h for follicles classification and mapping.
Participants/materials, setting, methods: Three ovaries of three different 8-week-old CD1 mice were fixed in 4% Paraformaldehyde and treated with Lugol's solution for 3 hr at RT. Ovaries were scanned with Skyscan 1172 (Bruker) using a 1.5 µm/pixel resolution. MicroCT sections were processed with Fiji ImageJ (NIH), and 3D rendering of follicles and blood vessels were obtained with Avizo-9 (Thermo Fisher Scientific). ANOVA and Bonferroni *post-hoc*statistical analyses were performed with RStudio, considering data significantly different when $p<0.05$.
Main results and the role of chance: Using microCT we built the first *in silico*3D reconstruction of the tiny mouse ovary, identifying, mapping and counting follicles,from pre-antral secondary T4 (53.2+12.7 µm in diameter) to fully-grown antral T8 (321.0+21.3 µm), and the *corpora lutea*.MicroCTbrought up the main functional compartments of the growing follicle, i.e., granulosa and cumulus cells, the antrum, the zona pellucida, and the oocyte with its nucleus. Instead, primordial and primary follicles (T1–T3) could not be observed, perhaps due to the reduced size of their enclosed oocyte and to the absence of a well-formed zona pellucida around the germ cell. In addition, our analysis allowed the visualisation and 3D modelling of the main ovarian vasculature, from the largest vessel that enters the organ at the hilum site (~150 µm size in diameter)to smaller branches present in the medulla region (~35 µm).

These results show that each of the eight ovarian sectors, virtually segmented along the dorsal-ventral axis,houses an equal number of each follicle type, suggesting a simultaneous and homogeneous distribution of follicle recruitment all-over the cortex,and subsequent growth within the same ovarian region.
Limitations, reasons for caution: To strengthen the results, the number of ovaries/individuals analysed should be increased.
Wider implications of the findings: This 3D mapping of follicles and vessels could contribute our understanding of folliculogenesis dynamics, not only under normal conditions, but also during ageing, after hormones or drugs administration, or in the presence of ovarian pathologies.
Trial registration number: not applicable

O-158 The effect of circadian rhythm disruption due to constant light on ovarian and oocyte aging through possible relationship between PER2 and mTOR signaling pathway

G. Bora[1], T. Onel[1], E. Yildirim[1], A. Yaba[1]

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Study question: Does circadian rhythm disruption by constant light affect the ovarian morphology and function, and cause ovarian and oocyte aging through possible relationship between PER2 and mTOR?
Summary answer: We demonstrated that circadian rhythm disruption by light may cause ovarian and oocyte aging.
What is known already: Circadian rhythm regulates multiple physiological processes and PER2 is one of the core circadian rhythm components. Changes in light conditions may cause circadian rhythm disruptions. Light exposure at night may cause attenuation in PER2 mRNA and protein levels. Circadian rhythm disruptions are thought to be associated with reproductive diseases. mTOR signaling pathway functions in folliculogenesis and oocyte maturation in ovary. Also, it is associated with ovarian and oocyte aging.
Study design, size, duration: A total of 32 female Balb/c mice which enter estrous cycle were used in the study. Mice were randomly assigned to one of two groups as 12:12h L:D and 12:12h L:L. During the experiment, 12:12h L:D (control group) was housed in a 12:12h light:dark cycle and 12:12h L:L (experiment group) was housed in a constant light conditions 12:12h light:light for 1 week.
Participants/materials, setting, methods: We housed 12:12h L:D group in standard lightening conditions and 12:12h L:L group in constant light for one week. We performed food intake and body weight change analysis. We evaluated ovarian morphology, follicle counting analysis. We evaluated ZP3 and nitrotyrosine (NTY) expression for oocyte aging markers. We performed western blot for PER2, mTOR, p-mTOR, p70 S6K, p-p70 S6K, and Caspase-3 protein levels.
Main results and the role of chance: We demonstrated that circadian rhythm disruption caused alteration in their food intake and decrease in primordial follicle numbers and increase in atretic follicles ($p<0.05$). It caused increase in oxidative stress and decrease in ZP3 expression in oocytes ($p<0.05$). We showed decreased protein levels of PER2, mTOR, p-mTOR and p70 S6K ($p<0.05$).
Limitations, reasons for caution: The explanation of molecular mechanism underlying the relationship between circadian rhythm disruptions by light and ovarian function may lead the usage of circadian rhythm-based or light-based therapies currently using to treat some diseases on female reproductive system related diseases.
Wider implications of the findings: We conclude that constant light may reduce follicle reserve, cause follicles to go rapidly atresia and disrupt the oocyte quality, thus it may be a risk factor for female reproductive diseases such as premature ovarian insufficiency and early menopause.
Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 49: NUTRITION, LIFESTYLE & REPRODUCTIVE ENDOCRINOLOGY

30 June 2021 Stream 4 10:00 - 11:30

O-159 Prediction of weight loss and drop-out in a lifestyle intervention in women with pcos: A randomized controlled trial

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Study question: Which patient related determinants contribute to a ≥5% weight loss and drop-out?
Summary answer: Participating in the lifestyle treatment and a worse body image at baseline were significantly associated with ≥5% weight loss.
What is known already: In general, three-component interventions including diet, exercise, and cognitive behavioral therapy have shown to be effective at the long-term to achieve weight loss. In a lifestyle program for infertile women, higher external eating behavior scores and not receiving previous support by a dietician were associated with weight loss. In a short term lifestyle program for women with PCOS, weight loss was associated with better quality of life scores and attendance of study appointments. Little has been published about the potential role of PCOS characteristics, psychological and behavioral variables on the ability to achieve weight loss in this group of women.
Study design, size, duration: The present study is a longitudinal RCT to study the effectiveness of a three component 1-year cognitive-behavioural lifestyle intervention on weight loss in overweight/obese women with PCOS. A total of 183 participants were randomly assigned to three groups: 1) CBT provided by the multidisciplinary team or; 2) CBT provided by the multidisciplinary team and Short Message Service (SMS) or; 3) usual care: women are encouraged to lose weight through publicly available services (control group).

Participants/materials, setting, methods: Women with menstrual cycle disorders are systematically screened using a standardised protocol. Data of 183 women diagnosed with PCOS according to the Rotterdam criteria, a Body Mass Index above 25 kg/m² were included. All variables were measured at start and at three, six, nine and twelve months.
Main results and the role of chance: The multivariable mixed-effect logistic regression model showed that participation in the lifestyle treatment (HR 2.3, P=0.012) and a worse body image (FNAE) (HR 0.95, P=0.023) at baseline were significantly associated with ≥5% weight loss. Drop-out was predicted by participation in the lifestyle treatment (OR 0.2 P=0.003), additional short message service (OR 3.7, P=0.008), smoking (OR 0.3, P=0.22), drinking alcohol (OR 2.4, P=0.04), higher levels of androstenedione (OR 1.2, P=0.047). Also, women who achieved spontaneous pregnancies were more likely to drop-out (OR 0.09, P=0.002).
Limitations, reasons for caution: A limitation of our study is the high discontinuation rate we observed especially after 3 months of the intervention. Therefore a statistical method was chosen that included all available data even if participants dropped out during the study period.
Wider implications of the findings: A three-component lifestyle intervention program for obese women with PCOS is effective for weight loss. The group of women with a more negative body image should receive additional treatment before entering such a lifestyle intervention to achieve better results.
Trial registration number: Registered at the Netherlands National Trial Register with number NTR2450 on August 2nd, 2010.

O-160 The effect of 6-month nutritional intervention on the anthropometric, biochemical, and reproductive profile of Lebanese women with Polycystic ovarian syndrome

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[2]American University of Beirut, Medicine, Beirut, Lebanon ;
[3]University of Westminster, Faculty of Science and Technology, London, United Kingdom

Study question: This study aims to assess the efficacy of, design, and implement a public health nutrition intervention designed to enhance healthy eating and life style management among PCOS patients.
Summary answer: After 6 months, this intervention decreased initial body weight by 5%-10%, increased pregnancy rate by 70%, and significantly improved psychological, metabolic and endocrine profiles.
What is known already: Polycystic ovary syndrome (PCOS) is a common endocrinopathy among women in their reproductive age and is characterized by imbalanced hormones, irregular menses, and fertility problems. PCOS has been linked to obesity and insulin resistance in many studies and this association suggests that a weight management intervention consisting of a lifestyle modification (LSM) program might improve the metabolic, reproductive and biochemical profiles of PCOS patients, especially that studies showed that weight loss among PCOS women reduces hyperandrogenism and thus improve ovulation.
Study design, size, duration: The study is a randomized control trial studying the effect of a 6 month weight management program with nutritional guidelines. 588 female participants attending the obstetrics/gynecology clinic, aged between 18 and 45 years old, were recruited. There were two obese/overweight (experimental and control) and two lean subgroups (experimental and control). Experimental groups received the intervention under study while the controls were given the usual programs (except for the non-PCOS lean group that received no intervention).
Participants/materials, setting, methods: Data on socio-demographic variables, nutritional status, physical activity, psychological and medical status were collected using a pre-validated questionnaire. Data on biochemical variables were collected from blood analysis. Data collection took place at baseline, after 3 and 6 months from intervention. Patients who were in the intervention groups were subject to nutritional education/counseling (following the proposed guidelines) as well as the structural weight loss program for obese patients.
Main results and the role of chance: After 6 months, PCOS women lost 8.2 kg (P=0.001), non-PCOS women lost 11.6kg among while controls gained weight. The biochemical, psychological and reproductive profile showed significant improvements among PCOS women. In fact, the percentage weight loss of 10% in the overweight/obese intervention group showed significant changes in some of the biochemical markers (total cholesterol FBS, CRP, LDL, and testosterone) (P<0.001). PCOS patients who got the intervention had a shorter time to ovulation, than those who didn't and had more average number of cycles during the 6 months study duration (P<0.001). Regarding the pregnancy rate for PCOS patients, it was shown that it increased significantly with intervention patients in all groups compared to controls (from 0% to 70% compared from 0% to 15 % respectively).
Limitations, reasons for caution: Limitations of the study includes inability to objectively assess patient compliance with innervation and inability to follow up with the participants for a longer period of time (12 months or more) for long term outcomes.
Wider implications of the findings: The findings of our study are in synchrony with previous studies that implied that nutritional and life style interventions improved PCOS outcome. This suggests the need for nutritional interventions and guidelines on additional to the traditioned gynecological treatment to improve PCOS outcome.
Trial registration number: Not applicable

O-161 Cumulative live birth rate after a freeze-all approach in women with polycystic ovaries: does the PCOS phenotype have an impact?

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[2]Crete University- Crete- Greece, Department of Obstetrics and Gynecology, Heraklion, Greece

Study question: Do cumulative live birth rates (CLBR) differ between PCOS phenotypes when a freeze-all strategy is used to prevent OHSS after ovarian stimulation (OS)?
Summary answer: When conventional-dose OS resulted in high response, a CLBR of ~ 70% was observed after "freeze-all" in women with PCOS, irrespective of their phenotype.
What is known already: Previous observational studies have shown that CLBR in women with PCOS who undergo assisted reproductive technologies (ART) may depend on their phenotype. When OS was performed with caution to avoid ovarian hyperresponse, CLBR was lower in women with a hyperandrogenic PCOS phenotype. However, when women with PCOS do exhibit hyperresponse and a freeze-all strategy is used, the impact of the PCOS phenotype on the clinical outcome of the ART cycle is unclear.
Study design, size, duration: This is a single-centre, retrospective cohort study including 422 women with polycystic ovary syndrome (PCOS) as defined by Rotterdam criteria or PCO-like ovarian morphology-only (PCOM) in whom a freeze-all strategy was applied after GnRH agonist triggering in the context of hyperresponse defined as ³19 follicles of ³11mm in their first or second IVF-ICSI cycle between January 2015 and December 2019 in a tertiary referral hospital.
Participants/materials, setting, methods: PCOS phenotype was based on hyperandrogenism (H), ovulatory dysfunction (O) and PCO-like ovarian morphology (P). Ovarian stimulation was performed with rFSH or HPhMG, adjusted to BMI. The primary outcome was cumulative live birth rate (CLBR) resulting from the transfer of all cryopreserved embryos from the same IVF-ICSI cycle. Patient and cycle characteristics and laboratory and clinical data were analysed. Data were analysed by multivariate logistic regression adjusting for covariates.
Main results and the role of chance: In total, 91/422 (21.6%) patients had PCOS phenotype A (HOP); 33 (7.8%) had phenotype C (HP), 161/422 (38.2%) had phenotype D (OP) and 137/422 (32.5%) had PCOM (n= 137). BMI, AMH and AFC were significantly different between phenotype groups (p<0.001), and highest in PCOS phenotype A. The type of gonadotropin used, as well as the mean daily and total stimulation dose were comparable for all groups. The mean number of retrieved oocytes was comparable among groups (22.4±10.8 for phenotype A, 21.4±7.1 for phenotype C, 20.4±7.8 for phenotype D and 22.2±9.7 for PCOM; p= 0.46). The mean number of embryos available for vitrification differed significantly (4.4±3.7, 5.7±3.4, 5.7±3.4 and 5.2±3.6, respectively; p= 0.005). Following the first frozen embryo transfer, LBR was comparable among groups (41.5%, 43.3%, 49.3% and 38.5%, respectively; p=0.31). Unadjusted CLBR was also similar (69.2%, 69.7%, 79.5% and 67.9%, respectively; p= 0.11). The multivariate logistic regression model adjusting for age, BMI,

number of oocytes and embryo stage (cleavage vs. blastocyst stage) confirmed that the PCOS/PCOM phenotype did not have any impact on CLBR (OR 0.80, CI 0.28-2.29 (phenotype C); OR 1.40, CI 0.67-2.90 (phenotype D); OR 0.65, CI 0.31-1.34 (PCOM); p= 0.1, with phenotype A as reference).
Limitations, reasons for caution: These data should be interpreted with caution as the retrospective nature of the study holds the possibility of unmeasured confounding factors. The results cannot be generalised to all ART cycles in women with polycystic ovaries as they pertain to those cycles where OS leads to hyperresponse.
Wider implications of the findings: In subfertile women with PCOS eligible for ART, hyperresponse after OS confers excellent cumulative live birth rates when a freeze-all strategy is used, eliminating unfavourable clinical outcomes that had previously been observed in hyperandrogenic PCOS women after mild OS targeting normal ovarian response and fresh embryo transfer.
Trial registration number: not applicable

O-162 Metabolic syndrome prevalence and severity during a randomized controlled three-component lifestyle intervention in women with PCOS

A. Dietz de Loos[1], G. Jiskoot[1,2], A. Beerthuizen[2], J. Van Busschbach[2], J. Laven[1]

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[2]Erasmus Medical Center, Psychiatry- Section Medical Psychology and Psychotherapy, Rotterdam, The Netherlands

Study question: What is the impact of a three-component lifestyle intervention on the prevalence and severity of metabolic syndrome (MetS) in women with polycystic ovary syndrome (PCOS)?
Summary answer: This three-component lifestyle intervention was more successful in improving metabolic health in reproductive-aged women with PCOS compared to minimal treatment.
What is known already: Women with PCOS have increased risk of MetS, and both PCOS and MetS are associated with excess weight. Moreover, obesity exacerbates many of the metabolic abnormalities associated with PCOS. Multicomponent lifestyle interventions are the first line treatment to improve weight. Previous studies in women with PCOS have described improvements in waist circumference, cholesterol, low-density lipoprotein and fasting insulin after (one-, or two-component) lifestyle interventions. However little is known about changes in the prevalence of MetS, continuous MetS severity z-score (cMetS z-score), different metabolic parameters and the effects of changes in weight per se after three-component lifestyle interventions.
Study design, size, duration: An randomized controlled trial (RCT) was performed and participants were either assigned to a one-year three-component (cognitive behavioural therapy, diet, exercise) lifestyle intervention (LSI), with or without additional short message service (SMS) support (SMS+ and SMS- respectively), both receiving 20 group sessions, or to care as usual (CAU, control) which consisted of advice to lose weight by methods of their own choosing. Overall, 183 women were included between August 2010 and March 2016.
Participants/materials, setting, methods: Women diagnosed with PCOS according to the Rotterdam 2003 criteria, aged 18-38 years, having a wish to conceive and a BMI >25 kg/m² were included at the Erasmus MC, The Netherlands. Outcome variables were evaluated every three months and included anthropometric measurements, ultrasound and an endocrine assessment. Multilevel linear and logistic regression was applied for longitudinal analyses.
Main results and the role of chance: The cMetS severity z-score decreased more in the SMS+ group vs CAU after one year (-0.39, p=0.015). MetS changed with -21.6% (p=0.037), -16.5% (p=0.190) and +7.0% (p=0.509) within the SMS-, SMS+ and CAU group respectively. Moreover, a post hoc analysis on the prevalence of MetS for both LSI groups combined vs CAU resulted in a difference of -25.9% (p=0.046) after one year in favour of the LSI groups. Weight loss per se resulted in significant favourable effects on all metabolic parameters.
Limitations, reasons for caution: Dropout during lifestyle interventions is unfortunately a common phenomenon, and our RCT also suffered from considerable discontinuation rates which is a limitation. Therefore, we selected a statistical method (multilevel regression modelling) specifically designed to deal with such missing values.
Wider implications of the findings: These findings confirm that three-component lifestyle interventions aiming at a 5-10% weight loss should be recommended for all women with PCOS in order to improve metabolic health during their reproductive lifespan.
Trial registration number: NTR2450

O-163 Hyperandrogenaemia in early adulthood is an independent risk factor for abnormal glucose metabolism in later life

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Study question: What is the role of androgen excess as a contributing factor to insulin resistance and abnormal glucose metabolism (AGM) in women?
Summary answer: There was a positive association between early adulthood hyperandrogenaemia with AGM. Serum SHBG levels could help identifying women at risk for disordered glucose metabolism.
What is known already: It is commonly recognised that insulin resistance induces compensatory hyperinsulinaemia which promotes ovarian androgen secretion. Studies in rodents have also suggested that testosterone causes prolonged activation of androgen receptor in pancreatic islet β-cells, inducing insulin hypersecretion and eventually secondary β-cell failure, thus predisposing to type 2 diabetes (T2D). However, the exact physiology behind the association between androgens, insulin resistance and T2D in women is not well understood. Many previously published studies are limited by cross-sectional study design, unrepresentative clinic populations, as well as variying steroid hormone measurement methods and definitions of androgen excess.
Study design, size, duration: A prospective longitudinal population-based cohort (n=5,889) to investigate whether serum levels of testosterone (T, measured using LC-MS/MS) and free androgen index (FAI) at ages 31 and 46 associated with AGM at age 46 years. After exclusion of pregnant women, users of hormonal intrauterine device, contraceptive pills, hormone therapy, minipills and statins, there were 4,421 women at age 31 and 4,457 women at age 46. At age 46 a two-hour OGTT was performed in 2,780 women.
Participants/materials, setting, methods: Serum fasting glucose and insulin, insulin resistance (HOMA–IR) and pancreatic β-cell function (HOMA–B) assessments were performed at ages 31 and 46. Elevated T levels (age 31: >2.3nmol/l; age 46: >1.7nmol/l) were defined according to the 97.5% percentile. T2D diagnoses were gathered from postal questionnaire at age 46, and verified and completed from the hospital discharge and national medication reimbursement registers. Impaired fasting glucose, impaired glucose tolerance or T2D were categorised as AGM.
Main results and the role of chance: At age 31, hyperandrogenic (HA) women displayed increased insulin resistance estimated by HOMA-IR (1.35±0.96 *vs.* 1.03±0.44, P=0.05), increased insulin secretion estimated by HOMA-B (115.05±34.67 *vs.* 99.25±25.47, P=0.006), and higher fasting insulin level (10.48±7.54 mU/l *vs.* 7.93±3.42 mU/l, P=0.034) compared with normoandrogenic (NA) women, after BMI adjustment. At age 46, HA women had comparable HOMA-B levels (98.04±60.03 *vs.* 96.27±65.89, P=0.93) but their fasting glucose (5.57±1.06 mmol/l *vs.* 5.37±0.77 mmol/, P=0.07) and glycated haemoglobin levels (37.47±7.83 mmol/mol *vs.* 36.18±4.99 mmol/mol, P=0.07) tended to be higher.
Women in the highest T quartile (Q4: odds ratio [OR]=1.77, 95%CI: [1.14–2.76]) and in the two highest FAI quartiles at age 31 (Q4: OR=3.61 [2.16–6.03] and Q3: OR=2.11 [1.24–3.59]) had increased risk for AGM at age

46, independently of BMI, when compared with women in the lowest quartile. Similarly, women in the two highest FAI quartiles at age 46 had increased risk for AGM (Q4: OR=2.91 [1.82–4.64]) when compared with women in the lowest quartile, after BMI adjustment. The three highest sex hormone-binding globulin (SHBG) quartiles inversely associated with AGM, both at ages 31 and 46, independently of BMI (at age 31: Q4: OR=0.38 [0.24–0.62], at age 46: Q4: OR=0.27 [0.17–0.44]).

Limitations, reasons for caution: We used only serum T as a marker of HA, even though other androgens, such as androstenedione and adrenal androgens have a place in the evaluation of androgenicity in women. Further studies of other large populations are needed to confirm our results.

Wider implications of the findings: This is the first longitudinal, general population based study to confirm a positive association between early adulthood hyperandrogenaemia with AGM in middle adulthood independently of confounding factors. Our results further suggest that SHBG levels could help to identify women at risk for AGM.

Trial registration number: NA

O-164 Associations between hypothyroidism and adverse obstetric and neonatal outcomes: a population study of 9.1 million births

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Study question: Does hypothyroidism confer an independent risk for adverse delivery and neonatal outcomes, based on analysis of the Healthcare Cost and Utilization Project-Nationwide Inpatient Sample database?

Summary answer: After controlling for confounders, women with hypothyroidism are at an increased risk of hypertensive disorders of pregnancy, preterm delivery, placental abruption, hemorrhage and caesarean section.

What is known already: Surprisingly, studies in the literature on maternal and neonatal complications of hypothyroidism in pregnancy are relatively small. The largest study to date included 184,611 pregnancies overall, with 7140 with hypothyroidism. Maternal hypothyroidism has been associated with multiple adverse pregnancy outcomes. These findings have not been confirmed in a large population database study.

Study design, size, duration: This is a retrospective study utilizing data from the Healthcare Cost and Utilization Project-Nationwide Inpatient Sample (HCUP-NIS). A cohort of all deliveries between 2004 and 2014 inclusively was created. Within this group, all deliveries to women with hypothyroidism formed the study group (n=185,073), and the remaining deliveries were categorized as non- hypothyroidism births and comprised the reference group (n=8,911,715). The main outcome measures were pregnancy and perinatal complications. Patients were included once per pregnancy.

Participants/materials, setting, methods: The HCUP-NIS is the largest inpatient sample database in the USA. It provides information relating to seven million inpatient stays per year, includes ~20% of hospital admissions, and represents over 96% of the American population. Multivariate logistic regression analysis, controlling for confounding effects, was conducted to explore associations between hypothyroidism and delivery and neonatal outcomes. According to Tri-Council Policy statement (2018), IRB approval was not required, given data was anonymous and publicly available.

Main results and the role of chance: Women with hypothyroidism were more likely to be older than 25 years, Caucasian, have higher household incomes, private insurance and deliver in an urban teaching hospital, as compared with the non-hypothyroidism obstetrical population (p<0.0001, all cases). After adjustment for all statistically significant confounders, women with hypothyroidism were more likely to suffer from gestational diabetes mellitus (aOR 1.43, 95%CI 1.38-1.47), hypertensive disorders of pregnancy: gestational hypertension (aOR 1.17, 95%CI 1.11-1.22) and preeclampsia (aOR 1.21, 95%CI 1.16-1.27) (all P<0.001)). They were more likely to experience PPROM (aOR 1.19, 95%CI 1.09-1.29) and preterm delivery (aOR 1.12 95%CI 1.08-1.17), and deliver by caesarean section (aOR 1.21, 95% CI 1.18-1.24 (all P<0.001)). Women with hypothyroidism more often developed chorioamnionitis (aOR 1.09, 95%CI 1.01-1.17, P=0.019), maternal infections (aOR 1.08, 95% CI 1.01-1.16, P=0.017), post-partum hemorrhage (aOR 1.07, 95%CI 1.01-1.13, P=0.012), disseminated intravascular coagulation (aOR 1.20, 95%CI 1.00-1.43, P=0.047), require blood transfusions (aOR 1.12, 95%CI 1.03-1.22, P=0.009), and hysterectomy (aOR 1.42, 95% CI 1.13-1.80, P=0.012) compared to the control group. [HB1] As for neonatal outcomes, small for gestational age and congenital anomalies were more likely to occur in the offspring of women with hypothyroidism (aOR 1.20, 95% CI 1.14-1.27 and aOR 1.34, 95% CI 1.22–1.48, both P<0.001).

Limitations, reasons for caution: This is a retrospective analysis utilizing an administrative database that relies on data coding accuracy and consistency.

Wider implications of the findings: Women with hypothyroidism were more likely to experience pregnancy, delivery and neonatal complications. We found an association between hypothyroidism and; hypertensive disorders, post-partum hemorrhage, transfusions, infections, preterm deliveries and hysterectomy, among other problems. This data from a population sized database confirmed the findings of the smaller studies in the literature.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 50: RESEARCH FROM AND FOR NURSES AND MIDWIVES

30 June 2021 Stream 1 11:45 - 12:45

O-165 Expert panel and evidence-based development of the Logbook for the Nurses and Midwives Certification programme of the European Society of Human Reproduction and Embryolog

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[10]Dancet- Eline, Fertility Centre, Leuven, Belgium

Study question: How was the Logbook for the Nurses and Midwives Certification programme of the European Society of Human Reproduction and Embryology (ESHRE) developed?

Summary answer: The Logbook for the ESHRE Nurses and Midwives Certification programme, which questions 56 roles, was developed based on an extensive literature review and expert opinion.

What is known already: The ESHRE Executive Committee established the Nurses and Midwives Certification Committee (NMCC) in 2012. Since inception (2015), the certification programme has been delivered annually, with the exception of 2020 because of SARS-CoV-2. One-hundred-fourteen nurses/midwives have obtained ESHRE certification (passing rate=72%) and the programme is now accessible to nurses/midwives globally. The Certification program aims (i) to recognise the extended role of nurses/midwives delivering fertility care and (ii) to expand their theoretical background. The pre-requisites for certification are (i) being educated to a bachelor level of education and (ii) completing a practice based Logbook to demonstrate supervision of professional experience.

Study design, size, duration: Between 2012 and 2014, the NMCC completed a systematic literature search for papers relating to clinical, non-clinical and extended roles of nurses and midwives in fertility settings. In addition, the NMCC invited a larger expert panel of European senior nurses and midwives to a meeting to discuss their needs and preferences regarding the certification programme and to survey the diverse roles performed by nurses and midwives in their country.

Participants/materials, setting, methods: The NMCC comprised four nurses/midwives, one clinical embryologist, and one medical doctor (both in advisory capacity). The Medline database was searched by entering a search string in PubMed combining (MeSH) terms related to reproductive medicine and nursing or midwifery. Opinion and empirical papers relating to roles of nurses/midwives in fertility settings were included. The surveyed expert panel included twelve nurses/midwives, representing Belgium, Denmark, Finland, France, Norway, Slovenia, Sweden, Turkey, Ukraine, and the United Kingdom.
Main results and the role of chance: A total of 49 papers, of which 24 empirical papers, were identified with the search string (n=47) and snowball strategy (n=2). The papers originated from 13 countries spread across Asia, Oceania, Europe, and North America. All twelve European senior nurses and midwives responded to the survey. Finally, 56 different roles were included in the Logbook. Forty-four roles were performed by nurses/midwives working in fertility settings according to the surveyed expert panel (n=18), the literature (n=8), or both the surveyed expert panel and literature (n=18). An additional twelve observations of laboratory procedures were added by the NMCC. Substantial variation in roles and responsibilities existed across the countries from which the evidence originated. Whereas a considerate proportion of roles were performed in at least five countries (n=16/56), a minority of roles were only performed by nurses/midwives in some countries (n=7/56). Eight specialist roles (e.g. embryo transfer) were performed independently by nurses/midwives in some countries, whilst in other countries, nurses/midwives merely had an assisting role. In addition to completing the Logbook, participants were expected to write two ethical cases according to a guideline, testifying to their ability to reflect as a senior nurse/midwife. From 2015, the content of the Logbook was further developed.
Limitations, reasons for caution: This abstract relates to the development and content of the Logbook and not the curriculum/educational material required for the theoretical exam of the Nurses and Midwives Certification programme. The NMCC continuously improves the Logbook and elements have been added and removed since its creation.
Wider implications of the findings: The review and survey illustrated the variation in roles and responsibilities of nurses/midwives across the studied countries, further highlighting the opportunity for professional development within fertility care. Further research is required to elicit the experience of certified nurses/midwives with the programme and its impact on their professional and personal development.
Trial registration number: Not applicable

O-166 A smartphone video clip on the patient journey to reduce patient's anxiety: a randomized controlled trial

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Study question: Can a smartphone video clip detailing the patient journey decrease the anxiety of women and men on the day of their first oocyte aspiration?
Summary answer: The video clip does not affect the anxiety of women but does reduce the anxiety of men on the day of couples' first oocyte aspiration.
What is known already: Infertility and in vitro fertilisation (IVF) decrease the personal wellbeing of women and men. Couples shared that this contributed to their IVF discontinuation despite a good prognosis and reimbursement of IVF. Previous longitudinal studies confirmed that pre-IVF anxiety is associated with IVF discontinuation. Limiting treatment anxiety is, therefore, relevant for fertility patients and clinics. Studies from the field of reproductive medicine examining the effect of preparatory information on anxiety suggest that focussed interventions seem more effective than complex interventions. Several randomized controlled trials (RCTs) found that preparatory information movies reduce anxiety for out-patient cardiology procedures in women and men.
Study design, size, duration: This monocentric RCT randomized (1:1 allocation; computerized) 190 heterosexual couples about to start their first IVF cycle between care as usual (i.e. preparatory information session 1-3 months before IVF) and care as usual combined with a novel intervention during a 24 months recruitment period (2018-2020). The novel intervention is a 5-minute smartphone video clip detailing the patient journey on the day of oocyte aspiration, which was sent to both partners the day before oocyte aspiration.
Participants/materials, setting, methods: Upon arrival at a private fertility clinic for their first oocyte aspiration women and men independently filled out the 'STAI-State anxiety inventory' and the 'infertility distress scale (IDS)' and evaluated the novel intervention, if applicable. A minority of randomized couples didn't comply with the standard IVF trajectory (n=27) or didn't fill out the questionnaires (n=8). The data of 155 couples (76-79/group, a-priori sample size calculation requested minimally 76/group) was subjected to a modified intention-to-treat analysis.
Main results and the role of chance: Women and men were on average 33 and 35 years old, respectively. Couples had a mean duration of infertility of 27 months and 63 of them (41%) had tried intrauterine insemination. The background variables were equally distributed between the intervention (IG) and control group (CG). The video clip did not affect women's anxiety on the day of oocyte aspiration (mean STAI-State score IG 42.7±8.1 vs CG 42.1±8.5, p=0.67). However, men who watched the video clip were significantly less anxious than men who did not watch it (mean STAI-State score IG 35.8±6.4 vs CG 38.2±7.6, p=0.04). Surprisingly, infertility-specific distress was higher among women and men who watched the video clip (mean IDS scores of 25.8±4.9 and 22.6±5.0, respectively), as compared to women (p=0.05) and men (p=0.02) who did not watch the video clip (mean IDS score 24.3±4.6 and 20.8±4.7, respectively). All women and men of the intervention group, except one woman, would recommend the video clip to friends and family going through IVF. The intervention and control group did not differ significantly regarding clinical pregnancy rate (31/76 vs. 29/79, p=0.60) or miscarriage rate (2/76 vs. 3/79, p=0.68) 12 weeks after their first oocyte aspiration.
Limitations, reasons for caution: Patients nor assessors were blinded and there was no attention control group. Selection bias is plausible although the participation rate was 89%. Assessing infertility-specific distress the day after watching the video clip was not optimal, as priming couples to feel infertility-specific distress short term is less problematic than longer term.
Wider implications of the findings: Providing additional procedural information is interesting for clinics as patients recommended the video clip and as it decreased men's anxiety. A follow-up study should examine whether the video clip's priming effect on infertility-specific distress lasts longer than only the day after and whether the video clip affects IVF discontinuation.
Trial registration number: NCT03717805

O-167 Sleep quality and affecting factors in women undergoing in vitro fertilization treatment

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Study question: Does in vitro fertilization (IVF) treatment affects the sleep quality of women and which factors reduce the sleep quality in different stages of this treatment?
Summary answer: The sleep quality decreased as the IVF treatment process progresses. Advanced age, prolonged treatment, economical concerns, stress ect. were factors that adversely affected women sleep.
What is known already: According to worldwide research, women receiving IVF treatment may suffer from physiological and psychological symptoms and even experience sleep disturbance. Sleep disturbances are thought to be frequent in women undergoing IVF despite minimal research of this hypothesis. The literature emphasizes the positive effect of quality sleep on oocyte health. Especially sleep hormone melatonin is effective in development of oocytes quality. During sleep, hormones such as melatonin and growth hormone can protect oocytes from the effects of harmful metabolites and improve oocyte quality. Routine evaluation of sleep quality and affecting factors may be an important issue in women receiving IVF treatment.
Study design, size, duration: It was longitudinal study with repeated measures. The sample consisted of 158 women from 218 eligible patients who received IVF treatment in two infertility centers in Izmir between December 2017 and March 2019. Treatment-related side effects, cancellation of embryo

transfer, and women could not be reached after embryo transfer were excludation reasons. According to the statistical analysis, the effect size was calculated as 0.496 and the statistical power as 100%.

Participants/materials, setting, methods: Data were collected via a structured questionnaires, "The Descriptive Data Form", "Pittsburgh Sleep Quality Index", "Visual Analog Stress Scale" and "Treatment Related Physical Symptoms List". The sleep quality and effecting factors were evaluated in three stages; the data related to pre-treatment period in beginning of IVF trial, in oocyte pick up (OPU) day induction-OPU process data and 1-2 days before pregnancy test, post embryo transfer data were collected.

Main results and the role of chance: The average sleep quality score was 6.96 in the pre-treatment period, 8.03 in the induction-oocyte pick up process, and 8.87 in the post embryo transfer ($p< 0.001$), the higher score it is evaluated as lower sleep quality. There was also a strong positive correlation between the stress scores and sleep quality before and during the treatment periods ($r=7.638$ $p< 0.001$, $r=0.672$ $p< 0.001$, $r=0.694$ $p< 0.001$). Among the descriptive features; advanced age, low education level, shift work, prolonged marriage duration, among infertility features; prolonged treatment, female and male factor, economic distress and sleep habit; such as being exposed to light were associated with poorer sleep quality ($p< 0.001$). Related to physical symptoms such as the abdominal distention, abdominal pain and breast fullness observed induction-OPU period ($t=4.79$ $p= .00$, $t=2.93$ $p= .00$, $t=2.77$ $p= .00$) and besides nausea and fatigue in post embryo transfer period where symptoms that significantly affect sleep quality ($t=9.38$ $p= .00$, $t=4.20$ $p= .00$, $t=4.06$ $p= .00$, $t=4.27$ $p= .00$, $t=4.33$ $p= .00$). According to results it was determined that women sleep quality decrease along with progress of IVF trial. Consequently health professionals should assess women's sleep quality and give support in most risky periods.

Limitations, reasons for caution: The fact that some women cannot be reached by phone after embryo transfer during the data collection process can be shown as the limitations of the study. At the same time, the subjective evaluation of sleep quality can be expressed as another limitation of the study.

Wider implications of the findings: There is a need for an experimental, randomized controlled study with objective evaluation of sleep outcomes. Therewithal examining the effect of sleep outcomes on the oocytes quality and pregnancy rates will guide clinical practise during IVF and other assisted reproductive treatments.

Trial registration number: There is no number

O-168 Chronic pelvic pain is the most troublesome endometriosis pain symptom for women's quality of life

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Study question: Which pre-operative endometriosis pain symptom is most troublesome for the quality-of-life of women assessed at different meta-levels?

Summary answer: Of five pain symptoms chronic pelvic pain is most troublesome or has the strongest correlation to women's overall quality-of-life and overall and endometriosis-specific health status.

What is known already: Endometriosis affects women's quality of life negatively, and its impact seems to depend more on women's symptoms than on their degree of endometriosis. Experts proposed to include 'the most troublesome symptom' and 'overall pain' as core outcomes but did not define how to assess these outcomes. It would be interesting to find out which pain symptom (i.e. assessed for presence and intensity) has most impact on women's quality-of-life assessed at different meta-levels, including: overall quality-of-life (depending on amongst others one's professional and relational life besides health), general health status and endometriosis-specific health status.

Study design, size, duration: A prospective survey addressed 277 adult women scheduled for diagnostic and/or therapeutic surgery in a University endometriosis clinic between October 2016 and November 2019. Women were reminded twice of our request to fill out the coded but anonymous questionnaire package assessing five pain symptoms (i.e. chronic pelvic pain, dysmenorrhea, dyspareunia, dysuria and dyschezia) and assessing quality-of-life at three different meta-levels.

Participants/materials, setting, methods: Women scored five endometriosis symptoms between 0 (no pain) and 10 (worst imaginable pain), combined into 'overall pain' (0-50). 'Overall quality-of-life' was assessed with the Linear Analogue Scale (LAS; the higher, the better). General and endometriosis-specific health status were assessed with the overall scores of the EuroQol-5D and the Endometriosis-Health-Profile-30 (the higher, the better). Pearson correlation coefficients between the six pain scores and three measures of quality-of-life were computed ($p=0.003$; 0.05/18 as Bonferroni correction).

Main results and the role of chance: A total of 227 women took part (participation rate=82%) and the data of 202 women (mean age 31±5 years old) with surgically confirmed endometriosis were analysed. In the previous six months, the majority of women reported chronic pelvic pain (56%), dyspareunia (55%), dysmenorrhea (56%) and/or dyschezia (58.4%), while only some reported dysuria (25%). Women's mean overall pain score was 20 (±12). Women's mean overall quality-of-life was 65 (±16). On average women rated their general health status to be 62 (±18) and their mean endometriosis-specific health status was 53 (±18). Only the pain symptom chronic pelvic pain was correlated ($p<0.001$) to assessments of quality-of-Life at all three meta-levels. The correlation for endometriosis-specific health status was large ($r= -0.574$), while the others were medium ($r= -0.343$ & $r= -0.324$). After taking account of the Bonferroni correction for multiple testing the remaining four pain symptoms only had a medium correlation ($p<0.001$) to endometriosis-specific health status ($r= -0.356$ – -0.265; $p<0.001$) and they were not correlated to overall quality-of-Life or general health status. Overall pain had a medium correlation ($p<0.001$) to Overall quality-of-Life ($r= -0.270$) and general health status ($r= -0.259$) and a strong correlation ($p<0.001$) to endometriosis-specific health status ($r= -0.529$).

Limitations, reasons for caution: The majority of patients (60%) of the recruiting University endometriosis clinic had severe endometriosis (AFS-IV) and this study could be repeated in another setting. Directly asking women which pain symptom is most troublesome to them would be interesting besides exploring correlations between pain symptoms and quality of life.

Wider implications of the findings: Nurses, midwives and other health care professionals should devote attention to chronic pelvic pain during the anamnesis and women-centred care as this pain symptom is most troublesome for women's quality of life. Whether surgery decreases chronic pelvic pain and overall pain is currently followed-up in the studied prospective cohort.

Trial registration number: Not applicable

INVITED SESSION

SESSION 51: ENDOMETRIOSIS AND INFERTILITY. THE SILENT DISEASE

30 June 2021 Stream 2 11:45 - 12:45

O-057 The impact of diagnosis endometriosis as part of the fertility workup

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Abstract text

The impact of diagnosis endometriosis as part of the Fertility workup

Endometriosis is a multifaceted disease that may go from completely asymptomatic to a debilitating condition with severe pelvic pain complicated infertility. In the last few years, how we approach fertility in women with endometriosis has clearly changed, postponing definitive/radical surgery till the patient has completed her family. As a clear association exists with endometriosis and infertility, during the fertility workup it is one of the diseases to investigate, as it may have been missed in previous annual gynecologic checkups. Here we may face two problems: a) the stigma of diagnosis a young women with the label "endometriosis", as she may be under the pressure of a progressive disease that may or may not affect her quality of life, and b) if the diagnosis of endometriosis is positive, how this may affect the decision making process during the fertility journey.

In this lecture we will discuss the difficulties of early diagnosis of endometriosis, why most of the previous test have failed, and the new opportunity that miRNAs seem to offer. Once endometriosis is diagnosed –early or late stages- how this may affect spontaneous chances of pregnancy, ovarian reserve, oocyte and embryo quality, endometrial receptivity, and last but not least, time to pregnancy. Obviously, the prognosis changes over time, and women's age will be conditioning most of our decisions. We will try to identify whom to treat, to increase the absolute pregnancy rate, and when to treat, to reduce the time to pregnancy. Finally, we will discuss the opportunity of fertility preservation in this particular subgroup of women. Being aware of the potential damage that endometriosis by itself, or the associated ovarian surgery, may inflict on ovarian reserve make these women more proactive for fertility preservation.

O-058 The impact of endometriosis on fertility treatment

E. Somigliana[1]

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Abstract text

Endometriosis can affect natural fertility by distorting pelvic anatomy and by causing local pelvic inflammation that affects the quality of the oocytes, the function of the tubes and the sperm capacity to fertilize. Two approaches can be foreseen, either removing the disease with the aim of restoring normality (surgery) or overcoming the disease by retrieving oocytes in the ovary and allowing their fertilization in *in vitro* conditions (IVF). Both therapeutic approaches seem rational and wise. However, despite this apparently simple clinical scenario, management of endometriosis-related infertility has engendered long-lasting and burning debates over the last two decades and, to date, no consensus has been reached. Robust scientific evidence is scant, actually limited to the demonstration that laparoscopy modestly increases the chance of natural conception.

In this debate, it is noteworthy that chances of pregnancy do not represent the unique stake. Other factors can play a role and deserves consideration. Considering the choice of fertility treatment, he most relevant are the following: 1) the impact of ovarian endometriomas and their removal on ovarian responsiveness, 2) the impact of endometriomas and endometriosis in general on oocytes quality, 3) the additional risks of oocytes retrieval in the presence of endometriosis (infections in particular) 4) the detrimental effects of endometriosis-related hydrosalpinx, 5) the impact of endometriosis on uterine motility and endometrial receptivity, 5) the confounding effect of endometriosis-associated adenomyosis. Unfortunately, for the vast majority of these concerns, evidence is not definite and physicians have to take decisions based on their clinical commonsense.

INVITED SESSION

SESSION 52: THE END OF DONOR ANONYMITY. A MATTER OF FACT

30 June 2021 Stream 3 11:45 - 12:45

O-059 Direct to consumer genetic testing is the reason why

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O-060 Ethical implications of the direct to consumer genetic testing. What should donors and recipients know?

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Abstract text

The growth in the use of direct-to-consumer-genetic testing (DTCGT) is having a major impact on sperm, egg and embryo donor conception (hereafter donor conception). DTCGT services include family history sites, e.g. Ancestry.com, and medical testing sites, e.g.23andme. Despite the many different motivations people have for using these services, it is now easier to search and find donor relatives, with donor-conceived people, recipients of donor gametes and embryos, and donors all using these services to make hitherto unlikely connections. Some individuals have found large numbers of donor-siblings, while donors have been traced by their adult donor offspring. DTCGT can also reveal unexpected origins with the numbers of people finding out they are donor-conceived through DTCGT rapidly increasing. For example, one woman discovered she was donor conceived after using 23andme to assess her risk of breast cancer, an eventuality she had never anticipated when she decided to take th**at test.**

The increasing use of DTCGT has led to claims that donor anonymity is dead and we are entering a new era where the possibilities of finding our genetic relatives and extended family have dramatically expanded. These developments will produce new landscapes where different systems collide and interact, creating new ways of locating and finding donor relatives. In the UK for example, information on genetic relations, donors and donor siblings, is located within two very different systems: 'official' regulatory systems, such as central registers of information held by government bodies such as the Human Fertilisation and Embryology Authority's (HFEA) registers; and emerging digital online systems, of DTCGT.

This paper will explore how these new developments interact with existing ways of finding out information about donor relatives and consider the ethical and legal issues and challenges for fertility practice.

POSTER DISCUSSION

SESSION 53: ANDROLOGY POSTER DISCUSSIONS

30 June 2021 Stream 4 11:45 - 12:45

P-050 The effectiveness of the platelet-rich plasma treatment of men with severe oligoasthenoteratozoospermia

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Study question: To evaluate the effect of platelet-rich plasma (PRP) testicular injections on spermogram parameters of men with severe oligoasthenoteratozoospermia (OAT).

Summary answer: The PRP testicular injections have beneficial effects on spermatogenesis and enhance sperm concentration and motility in infertile men with OAT.

What is known already: The use of PRP therapy in assisted reproductive technologies is debatable. Despite the recent evidence of its positive effects in promoting endometrial and follicular growth, data from clinical studies are limited. There are only a few papers on the effectiveness of PRP therapy in the treatment of male infertility and sexual dysfunction. In more detail, the influence of PRP on spermatogenesis was carried out only on experimental animals. Although the mechanisms of its action have not yet been clarified, it is assumed that PRP, containing many biologically active molecules, realizes its effect through the tissue regeneration and cell proliferation.

Study design, size, duration: This prospective study included 68 men (34.6±5.2) years old with severe OAT (≤4 million/ml, motility ≤30%, normal sperm morphology ≤1%) receiving hormonal and antioxidant (AO) therapy during 6 months before in vitro fertilization cycles. 33 of them were injected once with autologous PRP (0.5 ml in each testicle). Spermogram and testosterone level were analyzed before the treatment and in 3, 4 and 6 months after it.

Participants/materials, setting, methods: Sperm concentration, motility and morphology in ejaculate of 33 men of PRP group were compared with those in the group of 35 men without PRP within 6 months of starting the treatment. Total and free testosterone level were measured in blood serum. PRP was prepared by centrifuging the patient's own blood in the anticoagulant-containing

tubes. The final concentration of platelets in the obtained sample was 950.000 – 1.250 000 cells in 1 ml.

Main results and the role of chance: 4 months after the PRP injection, sperm concentration and motility increased in 18 of 33 men of the PRP group compared with the baseline (before the treatment) – 4.2 (1.0;6.9) vs 1.4 (0.1;3.4) mln/ml ($p<0.05$) and 36.7 (30.6;45.8) vs 17.7 (6.7;28.2) % respectively ($p<0.05$). The maximum increase in sperm motility (but not in sperm concentration!) was observed in 24 men in 6 months – 49.6 (39.6;56.4) % ($p<0.05$). Percent of morphologically normal spermatozoa in ejaculate slightly increased only in 12 men in that time period from 0-1 % to 1-2%. The total testosterone level was 2.4 times higher than the baseline (31.6±7.2 vs 13.2±4.3 nmol/l, $p<0.05$), the free testosterone level was 1.8 times higher (14.5±3.5 vs 7.9±3.0 pgl/ml, $p<0.05$).

Unlike the PRP group, in the group of men without PRP treatment, the sperm parameters did not changed compared with the baseline in 4 months after the starting hormonal and AO treatment. A significant increase of sperin concentration was observed only in 17 of 35 patients in 6 months. Sperm motility and percent of morphologically normal spermatozoa after the treatment did not differ from the baseline. Changes in the testosterone levels were similar to changes in PRP group.

Limitations, reasons for caution: Only young and middle-aged men were considered in the study. Large randomized controlled studies are required to confirm the PRP therapy efficacy and safety of f various fertility disorders. There are also no standardized protocols for PRP preparation.

Wider implications of the findings: PRP therapy may have great potential for the treatment of male infertility and improving spermatogenesis. Optimization of methods of PRP preparation and dosage of testicular injections can enhance reproductive outcomes in assisted reproductive technologies.

Trial registration number: not applicable

P-054 Pre-selected for an award: Apoptosis related-microRNAs in Oligoasthenoteratozoospermia and Azoospermia men may reveal novel study of freezing damage

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Study question: Could choosing of non-apoptotic spermatozoa by biological biomarkers such as microRNAs promote post-thaw fertilization ability?

Summary answer: Biological alterations in correlation with apoptosis and oxidative stress markers such as microRNAs may preserve the function and fertility of spermatozoa during cryopreservation.

What is known already: Biological changes of cryopreserved spermatozoa such as microRNAs against cryo-injury were investigated. It was presented that several sperm parameters such as motility and abstinence period can impact the percentage of post-thaw sperm survival. Recent study, reported that microRNAs related to process of motility, sperm structure and apoptosis were associated with different expression after cryopreservation. More comprehensive study needed to fully mention the effect of microRNAs and their correlations with other biomarkers in cryopreservation.

Study design, size, duration: Our study was performed on 58 men who were 24-40 years old. Their ejaculated samples were classified as sever (concentrations less than 5 million sperm/mL) Oligoasthenoteratozoospermia (SOAT), mild (concentrations 5 million – 10 million sperm/ mL) Oligoastheno teratozoospermia (MOAT), obstructive azoospermai (OA), Non obstructive azoospermai (NOA) (absence of spermatozoa in the semen) and normal group (concentrations more than 15 million sperm/ mL). Then each sample was grouped into fresh and cryopreserved one.

Participants/materials, setting, methods: Density Gradient centrifugation (DGC) was performed to obtain high quality sperm without round cells after freeze-thawing. Biopsy of testicular tissue was prepared after Testicular Sperm Extraction (TESE) surgery. Then biological biomarkers were examined before and after cryopreservation including microRNA-122 (miR-122), miR-383, miR-15b, miR-184, miR-34c and target genes such as P53, Caspase9 and CYCLIN D1, using Quantitative real-time polymerase chain reaction (RT-PCR). Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and malondialdehyde (MDA) using imaging multi-mode reader.

Main results and the role of chance: There was a significant reduction in sperm total motility and morphology in Cryopreserved-infertile groups (MOAT and SOAT) compared with the Fresh-infertile groups. Decreased level of GPx activity was associated with increased concentration of MDA during freeze-thawing procedure in oligoasthenoteratozoospermia. Also increasing levels of SOD, and DNA fragmentation were showed. Our data demonstrated that reduction of CYCLIN D1 in MOAT-Cryopreserved ($P=0.0174$) and NOA-Cryopreserved ($P=0.0001$) groups were considerable compared with their fresh ones. We observed high level of Capase9 and in cryopreserved groups ($P=0.01$).The expression of miR-34c was increased significantly in NOA-Cryo ($P=0.0064$), and OA-Cryo ($P=0.0441$) in comparison with their fresh groups. The expression of miR-184 ($P=0.0275$) was enhanced in NOA-Cryo as compared to NOA-Fresh. Quantitative RT-PCR demonstrated meaningful decrease level of miR-383 expression in SOAT-Cryopreserved as compared with SOAT-Fresh ($P=0.0223$). On the other hand, expression level of miR-383 was increased in NOA group significantly ($P= 0.0437$) and in OA group non-significantly during freezing. There was non-significant decrease of miR-122 and miR-15b in MOAT and SOAT-Cryopreserved groups in comparison to their Fresh groups. We observed reduced expression of miR-122 ($P=0.0109$) and miR-15b ($P=0.0322$) in OA group after freezing. Also, there was meaningful increased level of miR-15b ($P=0.0234$) in NOA-Cryo compared with Fresh.

Limitations, reasons for caution: Because of the ethical principle, we can not obtain testicular samples from normal groups. So, we analyzed NO and OA groups with each other.

Wider implications of the findings: Our study documented that total motility can be interfered by microRNAs. This phenomenon effects on the total motility of post-thaw spermatozoa. Also the increase level of MDA may disturb microRNAs regulation in the infertile cases. These non-coding RNAs may be known as fertility biomarker to development of freeze-thawing strategies.

Trial registration number: 60961

P-064 Application of an artificial intelligence model for morphologic prediction of fertilization-competent human spermatozoa

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Study question: What is the role of artificial intelligence in selecting fertilization-competent human spermatozoa according to their morphological characteristics?

Summary answer: The established AI model in this study can be potentially used to select semen samples with superior fertilization potential in clinical settings.

What is known already: Defective spermatozoa-zona pellucida (ZP) interaction causes subfertility and is a major cause of low IVF fertilization rates. While ICSI benefits patients with defective spermatozoa-ZP binding, a standard method to identify such patients prior to conventional IVF is lacking. The application of artificial intelligence to sperm morphology analysis has become a topic of growing interest owing to the fact that the conventional assessment is highly subjective and time-consuming. Deep-learning, a core element of artificial intelligence (AI), incorporates the convolutional neural networks (CNN) to process all the data composing a digital image through successive layers to identify the underlying pattern.

Study design, size, duration: The fertilization-competent spermatozoa were isolated according to their binding ability to the ZP. The ZP-bound and -unbound spermatozoa were collected for functional assays and to establish an AI model for morphologic prediction of sperm fertilization potential. Human spermatozoa (n=289) were isolated from normozoospermic samples. Human oocytes (n=562) were collected from an assisted reproduction program in Hong Kong. Sample collection has been ongoing and will continue until the end of this study in November 2021.

Participants/materials, setting, methods: Sperm-ZP binding assay was employed to collect ZP-bound and -unbound spermatozoa. The fertilization potential and genetic quality of the collected spermatozoa were evaluated by our established protocols. Diff-Quik- stained images of ZP-bound and -unbound spermatozoa were collected respectively for the establishment of an AI model. A novel algorithm for sperm image transformation and segmentation was

developed to pre-process the images. CNN architecture was then applied on these pre-processed images for feature extraction and model training.
Main results and the role of chance: Our result showed that the sperm-ZP binding assay had no detrimental effect on sperm viability when compared with the raw samples and unbound-sperm subpopulations. ZP-bound spermatozoa were found with statistically higher acrosome reaction rates, improved DNA integrity, better morphology, lower protamine deficiency and higher methylation level when compared with the unbound spermatozoa. A deep-learning model was trained and validated by analyzing a total of 1,334 and 885 of ZP-bound/ unbound spermatozoa to evaluate the predictive power of sperm morphology for ZP binding ability. Our newly trained AI-based model showed initial success in classifying the ZP-bound/ unbound spermatozoa according to their morphological characteristics with high accuracy of 85% and low computational complexity.
Limitations, reasons for caution: This sperm selection method requires micromanipulation and relatively long processing time to recover ZP-bound spermatozoa. In addition to limited availability, the use of human materials may result in interassay variations affecting the reproducibility of this method among laboratories.
Wider implications of the findings: In light of current findings, AI-based sperm selection method may provide high predictive values of sperm fertilization potential for clinical purposes. This method is particularly applicable to patients who had poor fertilization outcomes after conventional IVF treatments or those with high degree of defective sperm-ZP binding ability.
Trial registration number: not applicable

P-117 Pre-selected for an award: Bioinformatic analysis of NRF2 in the study of association of NRF2 variant and male infertility related to smoking status

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Study question: Could *Nrf2* polymorphism (-617C>A; rs6721961) and oxidative stress (OS)-induced changes of signature seminal plasma (SP) miRNAs related to Nrf2 provide possible biomarkers of male infertility?
Summary answer: -617C>A SNP is associated with infertility through sperm OS DNA damage and miR-582-5p and miR-20a-5p, differentially represented between spermatozoa of smokers-non-smokers, might regulate Nrf2/ARE axis.
What is known already: As an extrinsic factor causing OS, smoking decreases male infertility by causing sperm membrane damage and DNA fragmentation. Expression of proteins related to the antioxidant defense system and phase 2 detoxifying enzymes controlled mainly by Nrf2/ARE pathway components is vital in managing OS-induced DNA damage. miRNAs, which multiple of are produced abundantly in male germ cells throughout spermatogenesis, have been detected in SP and contribute to multiple biological processes related to male reproductive events. miRNA-expression alterations may be induced in response to OS and without involving DNA sequence changes, miRNAs can provide additional mechanism of regulating the *Nrf2* gene expression.
Study design, size, duration: Wild-type (WT) and SNP (-617) alleles in the *Nrf2* gene were studied in 100 infertile cases and 100 controls and their associations with seminal parameters in relation to smoking status were assessed. In infertile cases, sperm DNA damage level was determined and compared among *Nrf2* genotypes. Interactions between differentially expressed miRNAs (DEMIs) in response to smoking and Nrf2/ARE pathway components were visualized on a miRNA-mRNA regulatory network using CluePedia (v1.5.7) plugin of Cytoscape software (v3.8.2).
Participants/materials, setting, methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was utilized to genotype the *Nrf2* SNP (-617). DNA damages were analyzed by Comet assay. DEMIs were identified by a comprehensive bioinformatics analysis using the miRNA expression dataset GSE44134 downloaded from the GEO database. Predicted targets of DEMIs in smokers were identified by mirDIP portal. Known interactions between Nrf2 and its first neighbors were visualized after selecting STRING-actions, miRTarBase and miRecords validated miRNA source files from CluePedia panel.
Main results and the role of chance: There was significant difference for *Nrf2* polymorphism between fertile and infertile males. The A allele was detected more frequently in the patient group; ($P = 0.001$). The frequencies of the C and A alleles of the *Nrf2* were 62% and 38% in patients, and 78% and 44% in control group. The AA genotype was higher in the infertiles; 14% vs. 3% ($P = 0.001$). In smokers, sperm quality decreased significantly in AA genotype. The risk of DNA damage was highest with 224.58 AU in the AA genotype group, whereas it is the lowest with 164.56 AU in those carrying the CC genotype ($P < 0.005$). 21 differentially expressed miRNAs (including 7 downregulated and 14 upregulated in smokers) were identified. Among the upregulated DEMIs, miR-582-5p, miR-20a-5p, miR-573, miR-186-5p, miR-499a-5p were found to target the *Nrf2* mRNA, suggesting their usage as biomarkers capable of indicating the antioxidant ability of the male reproductive system. The interrelations between Nrf2/Nrf2 direct interactors and DEMIs revealed the regulatory role of hsa-miR-20a-5p in SQSTM1/p62-Keap1-Nrf2 axis linked to selective autophagy. hsa-miR-582-5p was found to regulate the JNK/Jun/caspase-3 pathway, previously shown to be activated in response to OS, in which JUN can activate or suppress the *Nrf2* expression.
Limitations, reasons for caution: Small number of cases while evaluating the effect of smoking weakens our ability to generalize the results. Including other coexisting factors and larger patient groups carrying other functional variants of *Nrf2* as well as confirming the results at the protein level would further strengthen the results of the study.
Wider implications of the findings: This study is the first to report -617C>A polymorphism in the *Nrf2* gene in the Turkish population and such a SNP may cause impaired fertility in men, especially in smokers, through oxidative metabolism. Considering these data may be valuable in determining risk groups.
Trial registration number: N/A

INVITED SESSION

SESSION 54: THE FUTURE OF FERTILITY PRESERVATION

30 June 2021 Stream 1 14:00 - 15:00

O-061 From monkey to man: The fertility of testis tissue grafts

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O-062 In vitro maturation of oocytes in connection with ovarian tissue cryopreservation

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Abstract text

Discussing fertility preservation (FP) in young cancer patients has become a key component of routine oncological health care. Although ovarian stimulation followed by oocyte cryopreservation has been recommended in cases where two to three weeks are available before the start of chemotherapy, ovarian tissue cryopreservation (OTC) is the preferred option when this timeframe is not available and when the potential gonadotoxic impact of cancer therapy is deemed moderate or severe, or in prepubertal girls. During ovarian tissue processing in the laboratory, cumulus-oocyte complexes can be identified. In vitro maturation and further vitrification of oocytes retrieved in ex vivo from the extracted ovarian tissue (ovarian tissue oocytes in vitro maturation; OTO-IVM) can be attempted to enhance the future reproductive options of the patient. Although the number of reported live births after OTO-IVM are limited, this experimental FP procedure has potential to become a standard appended procedure in conjunction with OTC. In cancer patients with haematological tumours and ovarian invasion, or patients with primary tumours of the ovary, ovarian tissue grafting may be contraindicated because of the risk of reintroducing malignant cells. Utilisation of vitrified oocytes after OTO-IVM may be the only hope for genetic offspring for these patients. Moreover, exogenous hormonal pretreatment is not required and COC can be recovered during

ovarian tissue processing in the majority of patients who undergo partial or total unilateral oophorectomy. Nevertheless, maturation rates after OTO-IVM vary and are generally lower compared to IVM of transvaginally harvested IVM oocytes; currently available IVM systems registered for clinical use will have to be adapted to accommodate the *in vitro* requirements of oocytes derived from extracorporeal ovarian tissue, and follow-up data are needed to assess the success rate and safety of this novel approach.

INVITED SESSION
SESSION 55: CSRM EXCHANGE SESSION
30 June 2021 Stream 2 14:00 - 15:00

O-063 PGT-M, for multi-system genetic diseases

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Abstract text
Reciprocal translocations (RecT) and Robertsonian translocations (RobT) are among the most common chromosomal abnormalities that cause infertility and birth defects. In 2017, the Reproductive Medicine Center of the first affiliated Hospital of Zhengzhou University reported a method named "Mapping Allele with Resolved Carrier Status" (MaReCs), which enables chromosomal ploidy screening and resolution of the translocation carrier status of the same embryo. Meanwhile, the first international healthy baby, where the chromosomal balanced translocation that can be transmitted to offspring was precisely blocked by "MaReCS ", was born in our center . Roche translocation can also delivery healthy babies. Therefore, MaReCs accurately enables the selection of translocation-free embryos from patients carrying chromosomal translocations.In addition, with regard to the monogenic disorders and relative cases, our center used Karyomapping-SNP and NGS technology for preimplantation genetic diagnosis, completed the first Huntington's disease patient in China and delivered a healthy embryos. NGS/Karyomapping PGD can be used to assist pregnancy for all genetic diseases with clear genetic patterns and pathogenic genes.
Trial registration number:
Study funding:
Funding source:

O-064 Artificial intelligence in embryo selection of IVF

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Abstract text
Some studies have discussed the use of artificial intelligence and machine learning in the assessment and selection of embryos for in vitro fertilization. Complete artificial intelligence acquired using CNN's dark box algorithm could be highly useful in assessing in embryos, though it could be difficult to perform the external validation necessary to confirm its value. But due to the inherent drawbacks in complete artificial intelligence assessing in vitro developmental embryos, such as lacking results of discard embryos, dislocations between computer scientist and embryologist, low explanatory values in dark box algorithm, here, we suggest training computers to recognize the target region (internal pellucid zone region) and the features of embryos, then continuously score the embryos starting at in vitro fertilization through the zygote to the blastocyst stage. Parameters suitable for use with various endpoints in treatment sequence could be found by AI. Further clinical studies should be performed to validate the parameters and AI needed.
Trial registration number:
Study funding:
Funding source:

O-242 Metabolic coupling and spermatogenesis

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Abstract text
The microenvironment of spermatogenesis mainly consists of Sertoli cells, Leydig cells, and peritubular cells. The traditional theory considers that spermatogenesis is regulated by hypothalamus-pituitary gland- gonadal axis. In the hypothalamus-pituitary gland- gonadal axis, the microenvironmental cells are mainly regulated by the hormones, which are secreted by the hypothalamus or/and pituitary gland. Meanwhile, Sertoli cells and Leydig cells also secrete related factors to feedback the functions of the hypothalamus and pituitary gland. With the development of research, it has found that metabolic disorders are closely related to the spermatogenesis. Some components involving in lipid metabolism pathways, including saturated fatty acids, cholesterol, and triglycerides, etc. affect the functions of Sertoli and Leydig cells through metabolic coupling pathway. The disorder function of Sertoli and Leydig impairs the microenvironment of spermatogenesis, which finally leads to spermatogenic failure. Current studies have found that the imbalance of lipid metabolism can affect the intestinal flora, which induces the changes of related metabolites, and finally leads to the occurrence of male infertility. Based on the current research, we regulated the lipid metabolism by Omega-3 and metformin in clinic, the activity of spermatogenesis can be remolded.
Trial registration number:
Study funding:
Funding source:

SELECTED ORAL COMMUNICATIONS
SESSION 56: NEW INSIGHTS IN OOCYTE BIOLOGY
30 June 2021 Stream 1 14:00 - 15:00

O-169 Conventional ovarian stimulation with gonadotropins depletes the developmental proteome of mouse oocytes, reducing their size and compromising their fetal yield.

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[5]*University of Tokyo, Department of Medical Genome Sciences- Graduate School of Frontier Sciences, Kashiwa- Chiba, Japan ;*
[6]*Graz University of Technology, Institute of Biomedical Informatics, Graz, Austria*

Study question: Does conventional ovarian stimulation with gonadotropins alter the molecular composition, size, and developmental fitness of superovulated mouse oocytes?
Summary answer: Ovarian stimulation perturbs 31% of developmental proteome vs. 2% of transcriptome, yielding smaller oocytes that form blastocysts with less primitive endoderm and diminished fetal yield.
What is known already: Prior mouse studies aimed to assess the impact of superovulation on oocyte and embryo quality provided variable results. Aberrations were observed in some studies but not in others, and were associated with a variable time spent in the oviduct until retrieval for *in vitro* culture. Although the natural ovarian cycle lasts 4 days in mice, the conventional stimulation protocol time spans 2 days. No genome-wide studies have been devoted yet to the global gene expression of superovulated mouse oocytes and derivative embryos, to determine if gene products accumulate to the same extent as in natural cycles.

Study design, size, duration: Approx. 1100 female mice were injected, half with serial equine and human chorionic gonadotropin, eCG and hCG, to induce superovulation; the other half were injected with saline as control. Both groups were mated to vasectomized or fertile males to obtain, respectively, metaphase II and fertilized oocytes. These were removed from the oviducts, and followed up *in vitro* to blastocyst, or *in vivo* to term after surgical transfer to naturally cycling females.
Participants/materials, setting, methods: B6C3F1 oocytes (n= ~16000) from superovulation (10 I.U. eCG+hCG) or natural ovulation were measured for diameter, and upon fertilization, they were cultured in KSOM(aa) to collect preimplantation stages for analyses (mass spectrometry; RNA sequencing; immunofluorescence for counting of trophectoderm, epiblast, and primitive endoderm cells). Embryos at the 4-cell stage were transplanted to naturally cycling females (8/female, 45 recipients). Results were compared between natural ovulation and superovulation with 48h (conventional) or 72h interval of eCG-hCG stimulation.
Main results and the role of chance: Preimplantation embryos of superovulated oocytes were affected in 31% of the proteins (893 / 2855) vs. 2% of the transcripts (482 / 21784), compared to natural counterparts (adj.P<0.05, Wilcoxon test). Gene set enrichment analysis of the perturbed proteome returned the top-terms 'thin zona pellucida' (ZP1, ZP2, ZP3) and 'abnormal inner cell mass apoptosis' (DAB2, STAT3). Microscope measurements verified a thinner zona pellucida (p = 0.077, Wilcoxon test) along with a smaller diameter (p<0.0001, Wilcoxon test) of superovulated oocytes, which gave rise to blastocysts deficient in primitive endoderm (p<0.013, Fisher's exact test). Since 529 of the 893 differently expressed proteins were depleted, we considered that ovarian stimulation provided insufficient time for protein accumulation. Increasing the eCG-hCG interval from 48 h (conventional) to 72 h restored oocytes' diameters, and improved their fetal output from 25% to 59%, compared to 54% of natural ovulation (15 embryo transfers per group). Conversely, oocytes lost part of their developmental potential to the micromanipulation-assisted reduction of their volume. This study provides evidence of an additional novel effect of exposure to gonadotropins on mouse oocyte quality, whose mechanism is mediated not by the stimulated genital tract, but by the time-dependent accumulation of proteins in oocytes.
Limitations, reasons for caution: This is an animal model study based on one mouse strain. Ovarian stimulation protocols differ between mice and humans. There can be yet other, more subtle or long-term differences between superovulated and naturally ovulated oocytes, than those described here. Proteome and transcriptome analysis cover much, but not everything.
Wider implications of the findings: There is a trade-off between oocyte quantity and quality in mice subjected to superovulation. Cytological and molecular deficits define a 'small oocyte syndrome'. Problematic is not so much the gonadotropin treatment, rather its timing. An evidence-based protocol for superovulation may be different from that used currently in mice.
Trial registration number: Not applicable

O-170 Predictive value of cytoplasmic granulation patterns during IVF in MII oocytes from young donors

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[6]Vienna University School of Medicine, Department of Obstetrics and Gynecology, Vienna, Austria

Study question: Do ooplasm granulation patterns of donor MII oocytes have similar predictive values for in vitro fertilization (IVF) outcomes as they have in older infertile women?
Summary answer: Ooplasm granulation patterns of donor MII oocytes are predictive for IVF outcomes in young oocyte donors even more pronounced than in older poor prognosis patients.
What is known already: Cytoplasmic granules had been noticed for years, with data mostly focused on central granulation. Dispersed granulations were mentioned but lacked analysis.
Study design, size, duration: A retrospective cohort study during 2017-2020.
Participants/materials, setting, methods: We investigated 776 fresh and 381 vitrified-thawed MII oocytes from carefully selected young donors (mean, 26.7±2.7; range, 21-35 years) and determined cytoplasmic granulation patterns during intracytoplasmic sperm injection as fine, central, uneven, dispersed and peripheral (see only in thawed oocytes). Fertilization, pregnancy and live-birth rates in fresh and thawed donor oocytes were analyzed
Main results and the role of chance: In fresh donor oocytes: 2PN rates significantly trended down from 96.3% to 90.7%, 89.2%, 66.7% from fine to central, uneven, dispersed granulations; overall pregnancy rates trended down from 48.8% to 29.0%, 19.0% and 6.4%, as did live birth rates (42.1%, 21.6%, 12.5%, 6.4%), from fine to uneven, central and dispersed granulations. Known-pregnancy and known-live-birth analyses showed similar findings. Thawed donor oocytes demonstrated similar trends, though with significantly worse outcomes than fresh oocytes. Peripheral granulation, unique to vitrification and thawing, always demonstrated the worst IVF outcomes. Interestingly, granulation patterns were mostly disassociated from morphologic embryo grades in fresh and thawed donor oocytes.
Limitations, reasons for caution: As a retrospective cohort study, some cases had to be excluded for lack of information. The scoring system may have diluted the real contribution of an oocyte when two or more embryos were transferred.
Wider implications of the findings: Ooplasm granulation patterns have predictive values for fertilization, pregnancy and live birth in IVF cycles, supporting integration of them into embryo selection, and suggesting that ooplasm granulation patterns reflect intrinsic features of oocytes that relate to oocyte quality, cytoplasmic maturity and developmental competence, but are largely independent of clinical co-variables.
Trial registration number: NA

O-171 Altered meiotic spindle morphology and composition in in vitro matured oocytes

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Study question: How does the meiotic spindle tubulin PTMs of MII oocytes matured *in vitro* compare to that of MII oocytes matured *in vivo*?
Summary answer: MII cultured *in vitro* present detyrosinated tubulin in the spindle microtubules, while MII oocytes matured *in vivo* do not.
What is known already: A functional spindle is required for chromosomal segregation during meiosis, but the role of tubulin post-translational modifications (PTMs) in spindle meiotic dynamics remains poorly characterized. In contrast with GVs matured *in vitro* within the cumulus oophorous, *in vitro* maturation of denuded GVs to the MII stage (GV-MII) is associated with spindle abnormalities, chromosome misalignment and compromised developmental potential. Although aneuploidy rates in GV-MII are not higher than in vivo matured MII, disorganized chromosomes may contribute to compromised developmental potential. However, to date, spindle PTMs morphology of GV-MII has not been compared to that of *in vivo* cultured MII oocytes.
Study design, size, duration: GV (n=125), and MII oocytes (n=24) were retrieved from hormonally stimulated women, aged 20 to 35 years old. GVs were matured to the MII stage *in vitro* in G-2 PLUS medium for 30h; the maturation rate was 68,2%; the 46 GV-MII oocytes obtained were vitrified, stored, and warmed before fixing and subjecting to immunofluorescent analysis. *In vivo* matured MII oocytes donated to research were used as controls.
Participants/materials, setting, methods: Women were stimulated using a GnRH antagonist protocol, with GnRH agonist trigger. Trigger criterion was ≥2 follicles ≥18mm; oocytes were harvested 36h later. Spindle microtubules were incubated with antibodies against alpha tubulin and tubulin PTMs (acetylation, tyrosination, polyglutamylation, Δ2-tubulin, and detyrosination); chromosomes were stained with Hoechst 33342 and samples subjected to confocal

immunofluorescence microscopy (ZEISS LSM780), with ImageJ software analysis. Differences in spindle morphometric parameters were assessed by non-parametric Kruskal–Wallis and Fisher's exact tests.

Main results and the role of chance: Qualitatively, Δ2-tubulin, tyrosination and polyglutamylation were similar for both groups. Acetylation was also present in both groups, albeit in different patterns: while *in vivo* matured MII oocytes showed acetylation at the poles, GV-MII showed a symmetrical distribution of signal intensity, but discontinuous signal on individual microtubule tracts, suggesting apparent islands of acetylation. In contrast, detyrosination was detected in *in vivo* matured MII oocytes but was absent from GV-MII. Regarding spindle pole morphology, of the four possible phenotypes described in the literature (double flattened and double focused; flattened-focused, focused-flattened, with the first word characterizing the cortex side of the spindle), we observed double flat shaped spindle poles in 86% of GV-MII oocytes (25/29) as opposed to 40.5% (15/37) for the in vivo matured MII oocytes (p=0.0004, Fisher's exact test). Further morphometric analysis of the spindle size (maximum projection, major and minor axis length) and the metaphase plate position (proximal to distal ratio, angle) revealed decreased spindle size in GV-MII oocytes (p=0.019, non parametric Kruskal- Wallis test).

Limitations, reasons for caution: Oocytes retrieved from hyperstimulation cycles could be intrinsically impaired since they failed to mature *in vivo*. Our conclusions should not be extrapolated to IVM in non-stimulated cycles, as in this model, the cumulus oophorus is a major factor in oocyte maturation and correlation with spindle dynamics has been inferred.

Wider implications of the findings: The metaphase II spindle stability compared to the mitotic or metaphase I meiotic one justifies the presence of PTMs such as acetylation and glutamylation, which are found in stable, long-lived microtubules. The significance of the absence of detyrosinated microtubules in the MII-GV group remains to be determined

Trial registration number: not applicable

O-172 Metabolic imaging of cumulus cells to predict embryo implantation potential

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Study question: Can non-invasive metabolic imaging detect variations in cumulus cell metabolic parameters associated with a viable pregnancy of the corresponding embryo?

Summary answer: Noninvasive metabolic imaging can detect differences in the cumulus cell metabolic signatures between embryos that led to a viable pregnancy and those that did not.

What is known already: Bidirectional metabolic cooperativity between the human oocyte and its surrounding cumulus cells is essential for the oocyte to acquire full developmental competency. However, the relationship between cumulus cell metabolism and oocyte viability is not well established. Metabolic imaging uses Fluorescence Lifetime Imaging Microscopy (FLIM) to non-invasively measure autofluorescence of the endogenous molecules, NADH and FAD+, which are essential coenzymes for cellular respiration and glycolysis. This technique enables quantitative information for these coenzyme concentrations and regarding metabolite enzyme engagement. We have previously shown that this technique is an effective tool for quantitatively measuring metabolic state of mouse embryos.

Study design, size, duration: Cumulus cell clusters (n=617 from 193 patients) were dissected from cumulus-oocyte complexes prior to insemination or ICSI, vitrified, warmed and their metabolic function assessed. We conducted a prospective observational study to evaluate to what extent cumulus cells from an oocyte that led to a viable pregnancy (presence of a viable fetus >7 weeks gestation) after transfer of the corresponding embryo metabolically differed from those that did not. We also evaluated the associations with embryo morphology.

Participants/materials, setting, methods: Cumulus cell metabolism was assessed non-invasively using FLIM to measure the autofluorescence of NADH and FAD+. Overall a single FLIM measurement provides a total of 8 metabolic parameters (4 for NADH and 4 for FAD+). An additional parameter, the Redox Ratio was also acquired (NADH intensity / FAD+ intensity). We used multilevel models to investigate the association of cumulus cell metabolic parameters with the morphology of the corresponding embryo and clinical outcome.

Main results and the role of chance: Of the cumulus samples analyzed, 75 corresponded to embryos that did not result in a viable pregnancy, and 24 that did so. Significant associations were observed between cumulus cell FAD+ fraction bound to enzyme (p =0.007), FAD+ long lifetime (p= 0.01) and FAD+ short lifetime (p <0.001) and the clinical outcome of the corresponding embryo. These significant associations held up after controlling for age. We used a support vector machine algorithm to distinguish between embryos that led to a viable pregnancy and those that did not. The optimum hyperplane derived from a support vector machine algorithm predicted whether a sample with random cumulus cell metabolic parameters will lead to a viable pregnancy or not with an accuracy of 80%. Embryo morphological assessments were stratified as excellent, good, fair and poor. We found no significant associations between cumulus cell metabolic signatures and embryo morphology evaluated on day 3. Significant associations of FAD+ short lifetime (p <0.001) and day 5 embryo morphology were found. However, these associations were not significant after controlling for age.

Limitations, reasons for caution: Although we observed significant variations in metabolic parameters, further studies with larger sample sizes are required. Despite our validation studies showing no significant effect of vitrification on cumulus cell metabolic parameters, analyses with fresh clusters are needed to confirm our results.

Wider implications of the findings: Noninvasive FLIM imaging detects metabolic variations of cumulus masses and their association with embryo viability. The ability to correlate metabolic measurements of cumulus clusters, in combination with embryo morphology assessments and patient clinical characteristics, with embryo fate paves the way for this approach to be used in a clinical setting.

Trial registration number: 5RO1HD092559-03

SELECTED ORAL COMMUNICATIONS

SESSION 57: THE ART OF MANAGING LOW OVARIAN RESERVE - TOO LITTLE TOO LATE?

30 June 2021 Stream 4 14:00 - 15:00

O-173 Decline in anti-Müllerian hormone (AMH) concentrations following radioactive iodine (RAI) treatment in women with differentiated thyroid cancer (DTC): a systematic review and meta-analysis

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Study question: Does radioactive iodine (RAI) treatment in premenopausal women with differentiated thyroid cancer (DTC) affects ovarian reserve, as

evaluated by anti-Müllerian hormone (AMH), antral follicle count (AFC) and follicle-stimulating hormone (FSH)?

Summary answer: AMH concentrations decreased at three, six and 12 months following RAI treatment in women with DTC, whereas no difference was observed regarding FSH.

What is known already: Thyroid cancer is the third most common type of female malignancy and DTC is the most common histopathological type. Thyroidectomy constitutes the mainstay of treatment for DTC, followed by administration of RAI, which acts as an adjuvant therapy to destroy residual cancerous thyroid tissue. However, the effect of RAI on ovarian reserve of these women, as assessed by AMH, AFC and FSH, remains controversial.

Study design, size, duration: A systematic review and meta-analysis was performed aiming to identify studies evaluating the effect of RAI treatment on ovarian reserve in women with DTC. For this purpose, a literature search in the electronic databases PubMed, Scopus and CENTRAL was carried out until 06/12/2020. The primary outcome measure was the effect of RAI on ovarian reserve, as evaluated by AMH, AFC and FSH.

Participants/materials, setting, methods: Studies were eligible if they included premenopausal women with DTC, treated with a single RAI dose and assessed for at least one marker of ovarian reserve repeatedly within 12 months post-RAI.Meta-analysis of weighted data was performed using random effects model. Results were reported as weighted mean difference (WMD) with 95% confidence interval (CI).

Main results and the role of chance: Four prospective eligible studies, published between 2005 and 2020, were eligible for the meta-analysis, evaluating a total of 154 women. The number of participants ranged from 24 to 50. The single dose of RAI used to treat DTC ranged from 50 to 150 mCi. AMH concentrations decreased at three (WMD -1.66 ng/ml, 95% CI -2.42 to -0.91, p<0.0001; I2 0%), six (WMD -1.58 ng/ml, 95% CI -2.63 to -0.52, p=0.003; I2 54.7%) and 12 months (WMD -1.62 ng/ml, 95% CI -2.02 to -1.22, p<0.0001; I2 15.5%) following a single RAI dose compared with baseline (three studies; n=104). With respect to FSH concentrations, no difference was observed at six (WMD +3.29 IU/l, 95% CI -1.12 to +7.70, p=0.14; I2 96.8%) and 12 months (WMD +0.13 IU/l, 95% CI -1.06 to +1.32, p=0.83; I2 55.2%) post-RAI compared with baseline (two studies; n=83). No data on AFC was available.

Limitations, reasons for caution: The small number of studies and patients included, as well as the lack of data on AFC may have compromised the validity of the conclusions drawn. Moreover, subgroup analysis according to female age was not feasible, due to the lack of relevant data.

Wider implications of the findings: The negative effect of RAI on ovarian reserve in premenopausal women with DTC, as indicated by the decreased AMH, should be confirmed by data on AFC, which are currently not available. These findings necessitate close monitoring of ovarian reserve in such women, counselling them regarding the need for fertility preservation.

Trial registration number: N/A

O-174 Individualized versus standard FSH dosing in predicted poor responders: an RCT

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Study question: Is there a difference in fertility outcomes between individualized or standard FSH dosing in women scheduled for IVF with an expected poor response?

Summary answer: In predicted poor responders (AFC<10) undergoing IVF/ICSI, individualized FSH dosing does not improve ongoing pregnancy rates as compared to a standard FSH dose.

What is known already: Poor responders usually lead to many detrimental effects on IVF outcomes due to low oocyte number and quality which in turn result in low pregnancy outcomes and an increased chance of cycle cancellation. Clinicians often individualize the FSH dose using ovarian reserve tests (ORT), including antral follicle count (AFC), basal FSH (bFSH), and anti-Mullerian hormone (AMH). However, it is unclear whether individualized FSH dosing improves clinical outcomes.

Study design, size, duration: Between March 2019 and April 2020, we performed a single-center, parallel, open-label RCT in women with an AFC<10. A total of 661 women were randomized either to start FSH dosing at 300IU/225IU or 150IU. The primary outcome was live birth attributable to the first ART cycle within 18 months of randomization. In this abstract, we report ongoing pregnancy rates. Live birth date will be available at the meeting.

Participants/materials, setting, methods: Women referred for their first IVF/ICSI cycle, <43 years of age, AFC<10 were approached. A total of 328 women were allocated to the individualized group and 333 women were allocated to the standard group. In the individualized group, women with AFC 1-6 were assigned to 300IU/day (n=122), while women with AFC 7-9 were assigned to 225IU/day (n=206). In the standard group, women were assigned 150IU/day. Outcomes were evaluated from an intention-to-treat perspective.

Main results and the role of chance: For ongoing pregnancy rate attributable to the first ART cycle for individualized versus standard dosing was comparable [52.44% vs 46.25%, relative risk (RR): 1.29 (95%CI, 0.94-1.74), P=0.11]. Biochemical pregnancy rate [62.50% vs 62.16%, RR: 1.01 (95%CI, 0.74-1.39), P=0.929], clinical pregnancy rate [59.45% vs 58.86%, RR: 1.02 (95%CI, 0.75-1.40), P=0.877] and multiple pregnancy rate [5.18% vs 5.12%, RR: 1.01 (95%CI, 0.51-2.02), P=0.971] also did not differ between individualized and standard dosing. There are 24 women who are ongoing pregnancy but do not reach live birth in the completed embryo transfer cycle. The individualized group reported less poor response (31.1% vs 48.7%: P<0.001), more obtained oocytes (6.80 ± 3.85 vs 5.28 ± 3.22; P <0.001), less embryos (3.76 ± 2.70 vs 3.16 ± 2.42; P=0.004), and less good quality embryos (2.61 ± 2.29 vs 2.21 ± 2.05; P=0.018). When outcomes were compared over the first embryo transfer, ongoing pregnancy rates were 39.0% (128/328) versus 37.2% (124/333), respectively [RR:1.08 (95%CI, 0.79-1.48), P=0.636], without differences in the other outcomes. There are 7 women who are ongoing pregnancy but do not reach live birth in the first embryo transfer cycle.

Limitations, reasons for caution: Due to the open-label character, potential selective canceling and small dose adjustments of standard dosing were allowed. This abstract reports on ongoing pregnancy. At the meeting, we will present live birth rates.

Wider implications of the findings: In women with predicted poor response, an increased dose does not increase ongoing pregnancy rates. A standard dose of 150IU/day is recommended in these women.

Trial registration number: ChiCTR1900021944

O-175 Impact of female chromosomal polymorphic variants on ovarian reserve markers and fertility treatments prognosis.

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Study question: Could the presence of chromosomal polymorphisms in women undergoing fertility treatments influence ovarian reserve, oocyte preservation or IVF clinical outcomes?

Summary answer: Polymorphic variants of chromosomes seem to adversely influence the Anti-Müllerian Hormone (AMH) serum levels and the post-thawing oocyte survival rate.

What is known already: Infertile couples have been reported to have a higher incidence of karyotype abnormalities than the general population, which could lead to lower fertility rates. However, few studies have demonstrated the controversial relationship between these karyotype alterations in women and the specific involvement of these variants and their combinations in an IVF cycle.

Therefore, there is a growing interest in categorizing chromosomal polymorphic variants and relating them to the subsequent evolution in ART cycles.

Study design, size, duration: Retrospective evaluation of a cohort of women undergoing IVF cycles in a private fertility center. The sample included 280 cycles performed between July 2017 and December 2020. The study explored the correlation between karyotype polymorphisms and IVF outcomes in terms of: Anti-Müllerian Hormone (AMH) level, Antral Follicle Count (AFC), Oocyte Maturity (MII), Oocyte Survival after Thawing (TS), Fertilization (FZ), Useful Embryos (UE), Biochemical (BP) and Clinical Pregnancy (CP), Miscarriage (M) and Live Birth (LB) rates.

Participants/materials, setting, methods: Women with karyotype performed before an IVF cycle. Chromosome analysis was carried out according to the International System for Human Cytogenetic Nomenclature guidelines (ISCN).

Only own eggs cycles were included, and testicular sperm cycles were excluded.

The normality of the distribution of the variables was assessed using the Shapiro-Wilk test. The association between IVF cycle parameters and the presence of polymorphisms was assessed by T-Student (parametric) or U-Mann-Whitney (non-parametric).

Main results and the role of chance: From a total of 280 IVF cycles, 198 met the inclusion criteria: Control Group (CG) with normal karyotype (94) and Study Group (SG) with presence of polymorphisms (104) were analyzed. Cycles with fresh (136) and warmed (62) oocytes were included. Mean female age was 37.66 ± 3.99 (CG) and 36.61 ± 3.72 (SG). The overall outcome rates were: 75.82% MII, 81.39% TS, 72.78% FZ, 49.07% UE on day 5, 21.82% BP, 78.18% CP, 14.53% M and 63.65% LB.

Statistically significant differences were found between the presence of polymorphisms and mean AMH serum level between CG (2.36 ng/mL) and SG (1.74 ng/mL) (p=0.04), especially when the type "qh+" was detected (1.01 ng/mL) (p=0.02). Furthermore, statistically significant differences were found regarding oocyte thawing survival rate, which decreased in the SG (78.94%) compared to the CG (93.69%) (p=0.02), mainly when the type "ps+" was detected (75.13%) (p<0.01).

No statistically significant differences were found between the presence of polymorphism and the AFC (p=0.25), MII (p=0.10), FZ (p=0.93) or UE (p=0.52) rate on day 5. In the same way, from 106 embryo transfers (ET) performed, no significant differences were found for BP, CP, M and LB rates (p>0.05).

Limitations, reasons for caution: Larger prospective studies including homogeneous cohorts are needed in order to corroborate our initial results.

Wider implications of the findings: Our findings may represent a practical tool in order to advise these patients about their reproductive success, especially, in terms of post-thawing oocyte survival prognosis. Therefore, it could be provided more personalized prognostic information before embarking on IVF treatments.

Trial registration number: Not Applicable

O-176 Secondary ovarian insufficiency (SOI) - a new infertility diagnosis

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Study question: Can, in contrast to primary ovarian insufficiency (POI), ovarian insufficiency also be caused as a secondary event (SOI)?**Summary answer:** Adrenal hypo-androgenism, caused by insufficiency in androgen production by the zona reticularis, may mimic POI in clinical presentation.

What is known already: A variety of small and large animal models have, especially at small growing follicle stages, conclusively demonstrated the dependence of normal follicle maturation on adequate testosterone levels, a process mediated via the androgen receptor on granulosa cells. Increasing clinical evidence, moreover, has demonstrated clinical improvements in oocyte numbers and oocyte quality following androgen supplementation at these stages in hypo-androgenic women with premature ovarian aging (POA). Whether adrenal hypo-androgenism in extreme cases can, however, also lead to complete ovarian insufficiency mimicking POI, is unknown.

Study design, size, duration: Based on FSH levels >40.0 mIU/mL and amenorrhea, we searched our center's electronic research data bank for patients who between 2016-2018 presented with a diagnosis of presumed POI.

Participants/materials, setting, methods: Among 78 POIs, 13 demonstrated low DHEAS (< 100 ug/dL), i.e., adrenal hypo-androgenism, 6 rejected egg donation and received DHEA (Fertinatal®, 25mg TID, Fertility Nutraceuticals, LLC, New York, N.Y., USA) and Coq10 (Ovoenergen®, 333mg TID,same manufacturer) before stimulation with 450IU FSH and 150 IU hMG (different manufacturers). All women were < age 41, demonstrated menopausal FSH (>40.0 mIU/mL), undetectable AMH, amenorrhea, > prior cycle cancellation, elevated SHBG, low total testosterone and low DHEAS (high DHEA/DHEAS).

Main results and the role of chance: Out of the 6 patients so stimulated, 5 demonstrated follicular responses following DHEA supplementation and 2/6 conceived spontaneously and delivered healthy offspring. One of these two is currently in treatment for another child.

Limitations, reasons for caution: To better understand adrenal control of ovaries via androgen production, further elucidation of endocrine signaling between adrenals and ovaries is required, including detection of unknown, though increasingly likely, feedback loops, considering that adrenals and ovaries share a primordium.

Wider implications of the findings: We confirmed existence of SOI due to adrenal hypoandrogenism, defined its phenotype, demonstrating that some patients with alleged POI actually exhibit SOI. Better pregnancy chances with adrenal SOI than POI, reemphasize the importance of correct differential diagnoses to avoid premature referrals of women into third-party egg donations

Trial registration number: N/A

POSTER DISCUSSION

SESSION 58: NURSING AND MIDWIFERY POSTER DISCUSSIONS

30 June 2021 Stream 1 15:15 - 16:30

P-467 Emotions, Thoughts, and Coping Strategies of Women with Infertility Problems on Changes in Treatment during Covid-19 Pandemic: A Qualitative Study

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Study question: What are the emotions, thoughts and coping strategies of women with infertility problems on changes in treatment during the COVID-19 pandemic?

Summary answer: Treatment-related procedures keep changing directions, exposing the women to high level of uncertainty. Changes in treatments may be perceived as threats to achieving parenting goals.

What is known already: Both infertility and the treatment process constitute a stressful experience. Literature reports that couples describe infertility as the most difficult challenge to overcome in their lives. In addition, it has been reported that women experience more anxiety, stress, and depression than men during this period. Societies and individuals affected by large-scale disasters, such as global pandemics, can develop stress-related disorders. Current data indicate that closure of fertility clinic during the COVID-19 pandemic was associated with a sharp increase in the prevalence of anxiety and depression among patients undergoing fertility treatments and was perceived as an uncontrollable and stressful event.

Study design, size, duration: The research was designed as a qualitative study. The data were collected from two Internet forums between October - December 2020. Blogs most frequently used by women with infertility in Turkey were simultaneously selected. The comments of 30 women were included.

Participants/materials, setting, methods: Data were screened by using the directed qualitative content analysis. After selecting the blog, emotions, thoughts, and coping strategies expressed by 30 women whose treatment was canceled due to the Covid-19 pandemic or who continued treatment during this period were included in the analysis. The themes created were adapted to Lazarus and Folkman's Transactional Model of Stress and Coping.

Main results and the role of chance: The thematic analysis of the expression of women with infertility problems in accordance with the Transactional Model of Stress and Coping stages of Lazarus and Folkman resulted in 4 themes: psychological changes, cognitive changes, changes in social life, and coping

strategies. Some women perceived changes in treatments positively, and stopping the treatments due to the uncertainty of the pandemic and its effect on pregnancy and the baby made them feel safe. The majority of women appraised the closure of fertility clinics negatively impacted their lives. They experienced despair, uncertainty, disappointment, anxiety, anger, sadness, and exhaustion from waiting. Also, some participants did not find it right to delay the treatments and felt that the healthcare personnel postponed the treatments to avoid infection. Women experienced feelings of anger, distrust, and threats toward the health authorities. Moreover, the women in our study stated that they were always at home due to the pandemic, far from friends and family, and therefore did did not feel need for self-care and considered themselves ugly. The expressions of women mostly include emotion-based coping strategies. They used activities such as praying, exercising, distracting, noticing the positive side of postponing, and stopping treatments during the pandemic, accepting, and meditating.

Limitations, reasons for caution: Clinics closed due to the pandemic or limited procedures caused fewer women to come to the clinics. At the same time, it is not accepted for anyone other than working in the clinic to come to the clinics for scientific studies. Therefore, comments of women have been reached through blogs.

Wider implications of the findings: It is believed that approaches based on Lazarus and Folkman's model helped the health professionals to determine potential stressors for women with infertility during the pandemic, and identified areas that required strengthening and improved personal coping strategies.

Trial registration number: not applicable

P-469 Period Tracker Applications – are they giving women accurate menstrual cycle information?

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Study question: Are period trackers giving women accurate information about their periods and ovulation?

Summary answer: The top 10 period trackers gave conflicting information on period dates, ovulation day and the fertile window.

What is known already: Period tracking applications allow women to track their menstrual cycles and receive a prediction for their periods. The majority of applications also provide predictions of day of ovulation and the fertile window. Previous research indicates applications are basing predictions on assuming women undergo a textbook 28-day cycle with ovulation occurring on day 14 and a fertile window between days 10 and 17.

Study design, size, duration: An audit of menstrual cycle apps was conducted on the Apple app store using menstrual cycle tracker/period tracker as the search terms. The top ten apps that followed the inclusion and exclusion criteria were analysed and used for this study. All apps had the ability to allow retrospective data entry giving future cycle predictions and fertile window, and nine of the apps predicted a day of ovulation.

Participants/materials, setting, methods: Five women's profiles for 6 menstrual cycles were created and entered into each app. Cycle length (CL) and ovulation day (OD) for the 6th cycle were; Woman 1 – Constant 28 day CL, 0D 16, Woman 2 – Average 23 day CL, OD 13, Woman 3 – Average 28 day CL, OD 17, Woman 4 – Average 33 day CL, OD 20 and Woman 5 – Irregular, average 31 day CL, OD 14.

Main results and the role of chance: For cycle length, the apps all predicted woman 1's cycles correctly but for women 2-5, the apps predicted 0 to 8 days shorter or longer than expected. For day of ovulation; for woman 1, no apps predicted this correctly; the apps ranged from day 13-15. For woman 2, 1 app was correct and overall the apps showed a lot of variation from day 8 to 13. For woman 3, no apps got it right, with a range of day 13-16. For woman 4, 2 apps got it right, but the apps ranged from day 13-20. For woman 5, no apps got right; the apps ranged from day 13-21. Irrespective of cycle length, 7 apps predicted a fertile window of 7 days in almost all cases; except 1 app that predicted 6 days for woman 2 and a different app which predicted 8 days for woman 4. For the remaining 3 apps, one always predicted a 10 day fertile window. One app predicted an 11 day fertile window in 4/5 women. One app predicted a 12 day fertile window in 4/5 women.

Limitations, reasons for caution: The five profiles created spanned a range of observed cycle characteristics, but many permutations are possible. A Monte Carlo type analysis could be conducted to examine these possibilities to provide more precise assessment of app performance, but as data had to be added manually into apps, this was not possible.

Wider implications of the findings: The apps do not use the same algorithm and show variation. The information given by these apps is not 100% accurate, especially for the day of ovulation and the fertile window which can only be predicted if using a marker of ovulation, such as basal body temperature or ovulation sticks.

Trial registration number: not applicable

P-470 Sex in the time of Covid-19: Examining the sexual behavior and sexual desire of female adults in Hong Kong

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Study question: How are sexual behavior and sexual desire of Hong Kong women affected during the Covid-19 pandemic?

Summary answer: The Covid-19 pandemic has a negative impact on the sexual life of adult women, in particular, single women who do not have a live-in partner.

What is known already: Since the beginning of the Covid-19 pandemic, there have been ongoing debates on whether lockdown measures would do more harm on individuals or families who are already living in fear of virus infection. Some studies have shown that despite social distancing and measures that limit contact and interaction, women, particularly those who are either married or have a stable partner, were found to be sexually more active and reported stronger emotional bonding with their partners during lockdown. This study attempts to examine any significant changes in sexual behavior and sexual desire of adult females in Hong Kong during the pandemic.

Study design, size, duration: This is a cross-sectional online study examining the sexual behaviors among female adults. The survey was conducted in Hong Kong between July and August 2020, in which the city has been locked down.

Participants/materials, setting, methods: Six hundred and two Chinese female adults (mean age = 32±7.09) were recruited through social media and community networks. Respondents completed the Desire Domain of the Female Sexual Function Index and self-reported frequency of sexual behavior before and during the Covid-19 pandemic. T-tests and ANOVAs were used to compare sexual behavior and sexual desire across demographic groups. Linear regression was conducted with sexual behavior and sexual desire as criterion variable and demographic characteristics as predictors.

Main results and the role of chance: Women reported significantly lower frequency of sexual behavior during the Covid-19 pandemic compared to previously ($t = 8.25$, $P < .001$). Less often did women feel sexual desire or interest during the pandemic ($t = 7.05$, $P < .001$) and a lower degree of sexual desire or interest was reported ($t = 11.16$, $P < .001$). During the pandemic, women who were married or cohabitated reported significantly more frequent sexual behavior than did single women with partners ($P < .01$), while the two groups were comparable in terms of the frequency and intensity of having sexual desire. Linear regression analyses showed a statistically significant reduction in frequency of sexual intercourse during Covid-19 with increasing age ($B = -.19$, $P < .001$), and being single with ($B = -.26$, $P < .001$) or without partner ($B = -.40$, $P < .001$), taking into account all other demographic characteristics. Single women reported significantly less often did they feel sexual desire or interest during Covid-19, while age ($B = -.26$, $P < .001$) and being single without a partner ($B = -.22$, $P < .001$) predicted significantly lower intensity of sexual desire during Covid-19.

Limitations, reasons for caution: Women with either primary or secondary education level are not adequately represented as recruitment was carried out via community network and social media platform, which are more likely to be more accessible by a population who is more tech-savvy and has more access to email.

Wider implications of the findings: We are still in the middle of the pandemic and there is still paucity of data illustrating its impact on sexual life. Current

findings could give insight to stakeholders to develop sexual health counselling services that address the negative effect on sexual intimacy arising from sexual behavioral change.
Trial registration number: Not applicable

P-472 Single mothers by choice - experiences of single women seeking treatment at a public fertility clinic in Denmark: A pilot study.

M.L. Steenberg[1], R. Sylvest[1], E. Koert[1], L. Schmidt[1]

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Study question: Are single women in fertility treatment stigmatised and what do they experience?
Summary answer: The women did not feel stigmatised. They experienced self-blame and negative thoughts about themselves, despite experiencing empowerment and receiving positive reactions from families and friends.
What is known already: Since 2007, medical doctors in Denmark have been permitted to offer medically assisted reproduction (MAR) also to single women. Denmark is a welfare state with a public health care sector providing MAR free of charge, 240 days of paid parental leave, and public full-time day-care. There has been an increase in the number of single women deciding to have children through the use of MAR. These women are referred to as 'single mothers by choice' (SMC), and they have been criticised for being selfish when raising a child without a father. Previous studies have shown how SMC can feel stigmatised.
Study design, size, duration: Semi-structured qualitative interviews at a public fertility clinic in Copenhagen, Denmark. Data collection took place between September and October 2020.
Participants/materials, setting, methods: The participants were single and childless women (N=6) undergoing MAR at the Fertility Clinic, Rigshospitalet in Copenhagen, Denmark. Five women received IVF and one received IUI. The women were between 30 and 40 years old and were all residents in the Capital Region of Denmark. The interviews were audiotaped, anonymised, and transcribed in full. Data were analysed using qualitative content analysis.
Main results and the role of chance: Single women did not differ from cohabiting women seeking MAR in relation to their experiences and attitudes towards motherhood. Four main themes were identified; (1) Experiences of single women seeking fertility treatment, (2) Emotions associated with becoming a single mother by choice, (3) The decision of becoming a single mother by choice, and (4) Family formation – a social interaction. The women would have preferred to have a child in a relationship with a partner and the shattered dream about the nuclear family has caused a wide range of experiences and emotions. The women did not feel stigmatised but they all had an awareness of the prejudices other people might have towards single mothers by choice. Hence, they were ready to defend their choice if necessary. On the other hand, they had received positive reactions and the process of becoming a single mother by choice was influenced by their social relations with family and friends. Despite their dream of the nuclear family the women choose to become SMC because motherhood was of such importance and they feared they would otherwise become too old to have children – the biological clock was ticking.
Limitations, reasons for caution: The participants were recruited from a public fertility clinic in the Capital Region of Denmark and may not be representative of all single women seeking MAR. Results might not be transferable to other countries with a different cultural context regarding the societal acceptance of different ways to establish a family.
Wider implications of the findings: This study contributes to the understanding of the experiences of single women seeking fertility treatment in a welfare state where there are no differences in the possibilities for different social classes to seek MAR in the public health care sector.
Trial registration number: N/A

P-473 Should couples be educated on how to try to conceive (TTC) before an infertility diagnosis? A comparative study of fertile, subfertile and infertile couples

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Study question: What sexual strategies do individuals TTC with different fertility status use?; What are the predictors of sexual dysfunction(SD) and frequency of intercourse(IF) when TTC?
Summary answer: TTC strategies with no evidence of effectiveness are the most used. Poor marital quality predicted SD, and female SD was a significant predictor of IF.
What is known already: It is well known that couples TTC have low fecundity knowledge. Previous evidence showed that after 12 months the frequency of intercourse decreases. After seeing a fertility specialist couples report a feeling of waiting time by attempting natural conception, which can be associated to evidence of an overestimation and excessive confidence in the success of fertility treatments. Existing guidelines recommend intercourse every other day, but no comparative studies exist up to date on what sexual strategies are used in different fertility status and what are the predictors of sexual frequency and sexual dysfunction when trying to conceive.
Study design, size, duration: This study is part of a randomized controlled trial on the effects of timed intercourse in psychosocial outcomes. Data was collected between July 2016 and November 2019 via an advertising strategy and obstetrics/gynecology centers. Inclusion criteria were: i) adult in a marital/cohabitation heterosexual relationship >1 year; ii) not knowing of any condition that can prevent pregnancy; iii) being actively TTC; iv) female age >22<42 years old; v) no previous children. Measurements were carried out online.
Participants/materials, setting, methods: Our final sample had 399 subjects (252 women). Participants rated the use of the following strategies: intercourse every other day (EOD), fertile week (FW), basal temperature, cervical mucus monitoring (CMM), ovulation predictor kits (OPK), and keeping legs elevated afterwards (EL). We also accessed psychological adjustment, relationship quality, SD and IF. Comparisons between groups were made by analysis of variance (ANOVA) and Chi-square tests, and logistic regression was used to determine predictors of SD and IF.
Main results and the role of chance: Participants were highly educated (72,8%), in the relationship for 9 years (±5.2), cohabitating for 5 (±3.6), and TTC for 2,5 years (range 0-16). Women were 33 years old (±4.4) and men 36 (±5.5). Regarding fertility status, 22.6% of participants were TTC <12 months, 22.8% >12 months but not diagnosed, 23.6% had a diagnosis but no treatment, and 31.1% had ART.

The most reported female strategy in all groups was EL, and the most never used was OPK. Differences were found in EOD, with significantly more women TTC <12 months that never used it, and more women with previous ART using it. Women who had ART are the ones who more frequently use FW and CMM comparing to other women (P>.05). In all groups, the majority reported IF once or twice/week. SD was found in 17.5% of women and 10,9% of men. Age (OR 0.91, 95%CI 0.85-0.97) and SD (OR 2.47, 95%CI 1.02-6.02) were the only predictors of low IF for women, with no significant findings for men. Poor relationship quality increased the risk of SD for both men (OR 0.11, 95%CI 0.03-0.40) and women (OR 0.46, 95%CI 0.03-0.40), and depression increased the risk of female SD (OR 1.24, 95%CI 1.06-0.46).
Limitations, reasons for caution: The cross-sectional nature of this study does not allow causal relationships to be determined. Further cohort studies allowing to assess differences as couples cross different fertility status are warranted. There are important predictors of SD that were not considered, specifically the comorbidity of diseases and pain.
Wider implications of the findings: Findings indicate that individuals TTC are misinformed, and that those using evidence-based sexual strategies are fertility patients. SD should be screened in patients TTC given that it might be an important predictor of IF. Couples might benefit from counselling to improve marital quality and consequently sexual functioning.
Trial registration number: NCT028140069

POSTER DISCUSSION
SESSION 59: EMBRYOLOGY POSTER DISCUSSIONS
30 June 2021 Stream 2 15:15 - 16:30

P-180 Bisphenols are present in culture media used for ART and cell culture

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Study question: Do plastic laboratory consumables and cell culture media used in human ART contain bisphenols?
Summary answer: Human embryo development media contained bisphenols close to the nanomolar concentration range while no release of bisphenols by plastic consumables was detected under routine conditions.
What is known already: The deleterious effect of the endocrine disruptor bisphenol A (BPA) on female fertility raised concerns regarding ART outcome. BPA was detected neither in media nor in the majority of plastic consumables used in ART, however it might have already been replaced by its structural analogs, including bisphenol S (BPS).
Study design, size, duration: Seventeen plastic consumables and 18 cell culture and ART media were assessed for the presence of bisphenols.
Participants/materials, setting, methods: Ten different bisphenols (bisphenol A, S, AF, AP, B, C, E, F, P, and Z) were measured using an isotopic dilution according to an on-line solid phase extraction / liquid chromatography/mass spectrometry method.
Main results and the role of chance: While all the plastic consumables of this study did contain bisphenols, none of them did release bisphenols under routine conditions. Moreover, 16 of the 18 cell culture and ART media assessed contained bisphenols, including 8 among the 10 media used in human ART. Five human ART media exhibited bisphenol concentrations higher than 0.8 nM and reached up to 3.2 nM (799 ng/L).
Limitations, reasons for caution: Further studies are required to investigate a greater number of ART media to identify less potentially harmful ones, in terms of bisphenol content.
Wider implications of the findings: As BPS has already been reported to impair oocyte quality at nanomolar concentrations, its presence in ART media, at a similar concentration range, could contribute to a decrease in the ART success rate. Thus far, there has been no regulation of these compounds in the ART context.
Trial registration number: Not applicable

P-181 Morphine regulates BMP4 growth factor and is involved in in-vitro early embryo development and PGCs formation.

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Study question: To elucidate if morphine can alter embryo development.
Summary answer: Chronic morphine treatment regulates BMP4 growth factor, in terms of gene expression and H3K27me3 enrichment and promotes *in-vitro* blastocysts development and PGC formation.
What is known already: BMP4 is a member of the bone morphogenetic protein family, which acts mainly through SMAD dependent pathway, to play an important role in early embryo development. Indeed, BMP4 enhances pluripotency in mouse embryonic stem cells (mESCs) and, specifically, is involved in blastocysts formation and primordial germ cells (PGCs) generation. Although, external morphine influence has been previously reported on the early embryo development, focus on implantation and uterus function, there is a big concern in understanding how environmental factors can cause stable epigenetic changes, which could be maintained during development and lead to health problems.
Study design, size, duration: First, OCT4-reported mESCs were chronically treated with morphine during 24h, 10^{-5}mM. After morphine removal, mESCs were collected for RNA-seq and H3K27me3 ChIP-seq study. To elucidate the role of morphine in early embryo development, two cell- embryos stage were chronically treated with morphine for 24h and *in-vitro* cultured up to the blastocyst stage in the absence of morphine. Furthermore, after morphine treatment mESCs were differentiated to PGCs, to elucidate the role of morphine in PGC differentiation.
Participants/materials, setting, methods: Transcriptomic analyses and H3K27me3 genome wide distribution were carried out by RNA-Sequencing and Chip-Sequencing respectively. Validations were performed by RNA-RT-qPCR and Chip-RT-qPCR.
Main results and the role of chance: Dynamic transcriptional analyses identified a total of 932 differentially expressed genes (DEGs) after morphine treatment on mESCs, providing strong evidence of a transcriptional epigenetic effect induced by morphine. High-throughput screening approaches showed up *Bmp4* as one of the main morphine targets on mESCs. Morphine caused an up-regulation of *Bmp4* gene expression together with a decrease of H3K27me3 enrichment at promoter level. However, no significant differences were observed on gene expression and H3K27me3 enrichment on BMP4 signaling pathway components (such as *Smad1*, *Smad4*, *Smad5*, *Smad7*, *Prdm1* and *Prmd14*) after morphine treatment. On the other hand, the *Bmp4* gene expression was also up-regulated in *in-vitro* morphine treated blastocyst and *in-vitro* morphine treated PGCs. These results were consistent with the increase in blastocyst rate and PGC transformation rate observed after morphine chronic treatment.
Limitations, reasons for caution: To perform the *in-vitro* analysis. Further studies are needed to describe the whole signaling pathways underlying BMP4 epigenetic regulation after morphine treatment.
Wider implications of the findings: Our findings confirmed that mESCs and two-cell embryos are able to memorize morphine exposure and promote both blastocyst development and PGCs formation through potentially BMP4 epigenetic regulation. These results provide insights understanding how environmental factors can cause epigenetic changes during the embryo development, leading to alterations and producing health problems/diseases.
Trial registration number: not applicable

P-186 Volumetric imaging provides insight into the 3D ultrastructural organization of maturing human oocytes

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Study question: Is volume electron microscopy suitable for high-resolution oocyte imaging in three dimensions (3D)?
Summary answer: Focused Ion Bean Scanning Electron Microscopy (FIB-SEM) allows 3D visualization and quantitative analysis of ultrastructural features in the large human oocyte volume.
What is known already: Transmission electron microscopy (TEM) has been traditionally used to study the fine morphology of female gametes. However, 2D micrographs provide only limited information about the topology of subcellular structures. Volumetric studies are needed to elucidate the 3D organization of complex oocyte cytoplasm.
Study design, size, duration: An academic study conducted in collaboration with an IVF clinic. Advanced 3D - ultrastructural analysis was performed on 9 human oocytes representing 3 stages of maturation (3 germinal vesicle (GV), 3 metaphase I (MI), 3 metaphase (MII) oocytes) collected from February 2018 to November 2019.
Participants/materials, setting, methods: Spare IVF oocytes, donated by 9 young egg donors (22-29 years), were cultured in vitro until they reached a defined developmental stage. Each oocyte´s meiotic status was determined based on the presence/absence of a prophase nucleus, a polar body, and a non-invasively detectable MI/MII spindle. Following standard TEM preparation, individual oocyte-containing resin blocks were coated with a thin carbon layer[JJ1] , mounted on the microscope stage, and subjected to FIB-SEM imaging.
Main results and the role of chance: FIB-SEM tomography provided an unprecedented view of the oocyte´s intracellular morphology. Automated serial scanning of newly exposed sample surface generated large stacks (120-1294 slices) of ultrastructural images with 40-100 nm z-resolution. The tomographic reconstruction of acquired datasets revealed the spatial arrangement of inner oocytes´ structures. The imaging protocol was optimized to ensure sufficient

image detail, minimal noisiness, and time-efficiency of large volume scanning. Comparison of oocytes fixed at different maturation stages confirmed previous TEM observations that the cortical region of GV oocytes is deprived of membraneous structures, and major organelle redistribution occurs during the MI phase. Semi-automated 3D image segmentation was employed to distinguish distinct organelle populations and evaluated their abundance. Subsequent quantitative analysis of volumetric data showed that the mitochondrion occupies ~ 5.27 % of MII oocyte volume. In conclusion, the volumetric imaging, followed by advanced image analysis, maximizes the amount of morphological data obtained from a single human oocyte.

Limitations, reasons for caution: The imaging procedure was pioneered on a small number of hormonally-primed oocytes, which failed to complete development in vivo. There is a trade-off between resolution, the size of the 3D volume, and imaging time. Block-face ion milling during FIB-SEM imaging inevitably results in sample destruction.

Wider implications of the findings: This proof-of-concept study opens up new possibilities to study the delicate architecture of scarce human oocytes. Enhancing our knowledge of the spatial organization of ooplasm is pivotal for developing experimental and therapeutical strategies involving oocyte microsurgery.

Trial registration number: not applicable

P-203 Applying artificial intelligence for ploidy prediction: The concentration of IL-6 in spent culture medium, blastocyst morphological grade and embryo morphokinetics as variables under consideration.

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Study question: Would it be possible to predict embryo ploidy by taking into account conventional morphological and morphokinetic parameters together with IL-6 concentration in spent culture medium?

Summary answer: Our artificial neural network (ANN) trained with blastocyst morphology, embryo morphokinetics and IL-6 concentration distinguished between euploid/aneuploid embryos in 65% of the testing dataset.

What is known already: The analysis of spent embryo culture media represents the protein and metabolic state of the embryo and could be a non-invasive method of obtaining information about embryo quality. The impact of the presence/absence of several proteins in embryo culture samples over clinical results has been widely studied. The IL-6 is one of the most mentioned protein for its effect on embryo development, implantation and likelihood of achieving a live birth. In this initial attempt, we examined the predictive value for euploidy of a model that took into account the concentration of IL-6 in the spent culture medium.

Study design, size, duration: This prospective study included 319 embryos with PGT-A results. Out of the total, 127 were euploid and 192 aneuploid embryos. Concentration of IL-6 in spent embryo culture media (collected on the day of trophectoderm biopsy-fifth/sixth day of development), morphokinetic parameters (division time to 2 cells-t2; to 3 cells-t3, to 4 cells-t4; to 5 cells-t5 and time of blastocyst formation-tB) and blastocyst morphological grade (according to ASEBIR criteria) were considered to predict the embryo ploidy.

Participants/materials, setting, methods: Embryos were cultured in EmbryoScope. The chromosome analysis was performed using next-generation sequence technology. The concentration of IL-6 was measured in 20µL of spent embryo culture media with ELISA kits. Morphokinetic parameters were automatically annotated and the blastocyst morphology was evaluated by senior embryologists based on blastocele expansion, inner cell mass and trophectoderm quality. All the embryos were divided into 70% for training, 15% for validating and 15% for testing our ANN model with MatLab®.

Main results and the role of chance: The general description for the euploid embryo population was the following: 2% of the embryos were graded as A, 71% were graded as B and 28% were graded as C; the means and standard deviations were 25.32±2.97 hours (h) for t2, 35.33±5.15h for t3, 37.30±5.43h for t4, 48.24±6.62h for t5 and 103.93±12.8h for tB; and the average of IL-6 concentration was 1.51±0.70 pg/ml. The general description for the aneuploid embryo population was the following: 1% of the embryos were graded as A, 48% were graded as B and 51% were graded as C; the means and standard deviations were 26.13±3.51h for t2, 36.70±4.29h for t3, 38.20±4.24h for t4, 49.86±6.89h for t5 and 107.10±8.29h for tB; and the average of IL-6 concentration was 1.47±0.71 pg/ml. Our ANN model showed a higher general success rate as we increased the variables considered in the final prediction of euploid embryos. The accuracy, sensitivity and specificity for the testing dataset were: 0.60, 0.12 and 0.87 with morphokinetic parameters; 0.63, 0.24 and 0.93 with morphokinetics and IL-6 concentration; and 0.65, 0.16 and 0.96 with morphokinetics, IL-6 concentration and blastocyst morphological grade.

Limitations, reasons for caution: The low sensitivity and high specificity achieved in our models indicated that they were more capable of detecting aneuploid than euploid embryos. As this was a preliminary study, the small number of embryos included in the test (n=48) was also a limitation.

Wider implications of the findings: The results showed that our model tended to classify the embryos as aneuploid. More euploid embryos would be necessary to train our model and achieve better results in the prediction of chromosomally normal embryos. Further studies with large number of embryos and additional variables could improve the non-invasive ploidy prediction.

Trial registration number: not applicable

P-240 Human extracellular vesicles (EVs) secreted by aneuploid embryos potentiate development of non-invasive PGT-A RNA biomarkers and stimulate MUC1 up-regulation in primary endometrial stromal cells (ESCs).

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Study question: Could EVs secreted by aneuploid embryos a) serve for development of RNA biomarkers for PGT-A and b) elicit a relevant transcriptomic response in decidualised ESCs?

Summary answer: Aneuploid embryo EVs a) contain *PPM1J*, *LINC00561*, ANKRD34C and TMED10 in differential abundance from euploid EVs and b) induce up-regulation of *MUC1* in decidualised ESCs.

What is known already: Embryo aneuploidy accounts for approximately 50% of all recurrent implantation failures in women >35 years old. PGT-A identifies euploid embryos to increase implantation probability but the technology is controversial as it requires an invasive embryo biopsy with an elusive long-term biosafety. The development of non-invasive methods to screen out aneuploid embryos is paramount. It is also critical to decode the embryo-endometrial dialog underlying implantation failure. We have previously reported that IVF embryos secrete EVs that can be internalised by ESCs, conceptualising that successful implantation to the endometrium is facilitated by EVs, which may additionally serve as biomarkers of ploidy status.

Study design, size, duration: Embryos destined for biopsy on days 5-7 for PGT-A were grown under standard conditions. Spent media (30µl) were collected from euploid (n=175) and aneuploid embryos (n=145) at both cleavage (days 1-3) and blastocyst (days 3-5) stage. Media samples from n=35 cleavage embryos were pooled in order to obtain five euploid and four aneuploidy pools. Blastocyst media were pooled to create one euploid and one aneuploid pool. ESCs were obtained from five women undergoing diagnostic laparoscopy.

Participants/materials, setting, methods: The study was realised at a research hospital. EVs were isolated from euploid and aneuploid Day3 pools with differential ultracentrifugation and EV-RNA sequencing was performed following the SMARTer Stranded Total RNA-Seq approach. ESCs were decidualised (E2:10nM, P4:1uM, cAMP:0.5 mM twice every 48 hours) and treated for 24 hours with 50 ng/ml euploid or aneuploid EVs extracted from blastocyst media. RNA sequencing was performed on ESCs following the Truseq RNAseq protocol.

Main results and the role of chance: Aneuploid cleavage stage embryos (n=4) secreted EVs that were less abundant in RNA fragments originating from the genes *PPM1J* (log2fc=-5.13, p=0.011), *LINC00561* (log2fc=-7.87, p=0.010)

and *ANKRD34C* (log2fc=-7.30, p=0.017) and more abundant in *TMED10* (log2fc=1.63 p=0.025) compared to EVs (n=5) from euploid embryos. Decidualisation *per se* induced downregulation of *MUC1* (log2FC=-0.54, p=0.0028) in ESCs as prerequisite for the establishment of receptive endometrium. The expression of *MUC1* transcript in decidualised ESCs was significantly increased following treatment with aneuploid compared to euploid embryo-secreted EVs (log2FC=0.85, p=0.0201).

Limitations, reasons for caution: The findings of the study may require validation utilising a second cohort of EVs samples.

Wider implications of the findings: This discovery that the RNA cargo of EVs secreted from aneuploid cleavage stage embryos is diverse from that of euploid embryos potentiates the development of non-invasive methodology for PGT-A. The upregulation of *MUC1* in decidualised ESCs following aneuploid embryo EV treatment proposes a new mechanism underlying implantation failure.

Trial registration number: NA

POSTER DISCUSSION

SESSION 60: IMPLANTATION AND EARLY PREGNANCY POSTER DISCUSSIONS

30 June 2021 Stream 3 15:15 - 16:30

P-365 Pre-selected for an award: Altered endometrial oestrogen-responsiveness and aberrant expression of cell-fate markers may contribute to the aetiology of recurrent pregnancy loss

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Study question: Do women with recurrent pregnancy loss (RPL) have an aberrant expression of oestrogen receptor- (ER) and cell-fate markers during the window of implantation (WOI) endometrium?

Summary answer: Women with RPL are found to have significantly altered levels of ER and Ki-67 in the WOI endometrium, possibly resulting in anti-proliferative and anti-angiogenic effects.

What is known already: RPL affects 1% of all women and has been associated with altered endometrial angiogenesis and proliferation when compared with the endometrium of healthy fertile women. RPL can be subcategorised into recurrent loss of anembryonic pregnancy, fetal loss (following evidence of a fetal heartbeat) and recurrent implantation failure (RIF). ER is the only oestrogen-receptor (ER) known to be expressed in the vascular endothelium of the endometrium and is the dominant ER during the WOI. It has an important role in endometrial regeneration and is proposed to regulate the angiogenic and vascular changes that occur in embryo implantation.

Study design, size, duration: This pilot case-control study took place at the Liverpool Women's Hospital and included 38 women; 29 who suffered RPL and 9 controls with proven fertility (≥2 healthy pregnancies). Of the RPL group, 9 had recurrent loss of anembryonic pregnancy, 10 had recurrent fetal loss and 10 had RIF. Endometrial samples were collected during the WOI (cycle day 22+/-2).

Participants/materials, setting, methods: To determine whether markers of endometrial cell proliferation and oestrogen-responsiveness are associated with RPL, we assessed the immuno-staining for ER , progesterone receptor (PR) and cell-fate marker Ki-67 in endometrial biopsies during the WOI using immunohistochemistry. A semi-quantitative immuno-staining score was used to assess the endometrial glands, stroma, luminal epithelium, perivascular and vascular endothelium compartments separately. Statistical differences between groups were calculated by non-parametric tests and significance level set at p<0.05.

Main results and the role of chance: During the WOI, the endometrial epithelium of women with RIF and recurrent anembryonic pregnancy loss showed significantly higher levels of ER when compared with fertile controls (p=0.01 and p=0.01, respectively). This may indicate an anti-proliferative process occurring at the site of implantation with very early pregnancy losses. In contrast, with women with recurrent fetal loss, a significantly lower level of ER was found within the vascular endothelium when compared with the fertile controls (p<0.01). This supports the theory that increased oxygen levels may compromise trophoblastic invasion, thereby leading to fetal loss.

The presence of Ki-67 (a marker of proliferation) was significantly lower within the vascular endothelium of all types of RPL: recurrent anembryonic loss (p=0.02), RIF (p=0.02) and recurrent fetal loss (p<0.01). These findings suggest ineffective endometrial angiogenesis in RPL, resulting in a suboptimal endometrial microenvironment.

PR was found to be significantly reduced (p<0.01) in the perivascular area of women with RIF versus fertile controls. Since decidualisation and preparation of the endometrium for a successful implantation is controlled by critical target genes downstream of PR, this alteration in PR may be an important feature of their defective endometrial phenotype.

Limitations, reasons for caution: Samples analysed were taken from the functional endometrium and therefore the results do not reflect the basalis. The WOI was identified using history and histological appearance, rather than timing with ovulation. Although we detected statistical significance, generalisation of the results requires further studies with larger sample size.

Wider implications of the findings: This data provides novel insight into the biological correlates of clinical types of RPL and suggests that specific alterations in the regulation of endometrial cell fate and oestrogen- responsiveness are associated with different types of RPL. This highlights possible new therapies for RPL, such as selective oestrogen receptor modulators (SERMs).

Trial registration number: Not applicable

P-377 Association between antinuclear antibodies and pregnancy prognosis in recurrent pregnancy loss patients

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Study question: Can antinuclear antibody (ANA) affect the subsequent live birth rate in patients with recurrent pregnancy loss (RPL) who have no antiphospholipid antibodies (aPLs)?

Summary answer: ANA did not affect the pregnancy prognosis of RPL women.

What is known already: The prevalence of ANA is well-known to be higher in RPL patients. Our previous study found no difference in the live birth rates of ANA-positive and -negative patients who had no aPLs. Higher miscarriage rates were also reported in ANA-positive patients compared to ANA-negative patients with RPL. The RPL guidelines of the ESHRE state that "ANA testing can be considered for explanatory purposes." However, there have been a limited number of studies on this issue and sample sizes have been small, and the impact of ANA on the pregnancy prognosis is unclear.

Study design, size, duration: An observational cohort study was conducted at Nagoya City University Hospital between 2006 and 2019. The study included 1,108 patients with a history of 2 or more pregnancy losses.

Participants/materials, setting, methods: 4D-Ultrasound, hysterosalpingography, chromosome analysis for both partners, aPLs and blood tests for ANA and diabetes mellitus were performed before a subsequent pregnancy. ANAs were measured by indirect immunofluorescence. The cutoff dilution used was 1:40. In addition, patients were classified according to the ANA pattern on immunofluorescence staining. Live birth rates were compared between ANA-positive and ANA-negative patients after excluding patients with antiphospholipid syndrome, an abnormal chromosome in either partner and a uterine anomaly.

Main results and the role of chance: The 994 patients were analyzed after excluding 40 with a uterine anomaly, 43 with a chromosome abnormality in either partner and 32 with APS. The rate of ANA-positive patients was 39.2 % (390/994) when the 1: 40 dilution result was positive. With a 1:160 dilution, the rate of ANA-positive patients was 3.62 % (36/994). The live birth rate was calculated for 798 patients, excluding 196 patients with unexplained RPL who had been treated with any medication.

With the use of the 1: 40 dilution, the subsequent live birth rates were 71.34 % (219/307) for the ANA-positive group and 70.67 % (347/491) for the ANA-negative group (OR, 95%CI; 0.968, 0.707-1.326). After excluding miscarriages

with embryonic aneuploidy, chemical pregnancies and ectopic pregnancies, live birth rates were 92.41 % (219/237) for the ANA-positive group and 92.04 % (347/377) for the ANA-negative group (0.951, 0.517-1.747).

Using the 1:160 dilution, the subsequent live birth rates were 84.62 % (22/26) for the ANA-positive group, and 70.47 % (544/772) for the ANA-negative group (0.434, 0.148-1.273).

Subgroup analyses were performed for each pattern on immunofluorescence staining, but there was no significant difference in the live birth rate between the two groups.

Limitations, reasons for caution: The effectiveness of immunotherapies could not be evaluated. However, the results of this study suggest that it is not necessary.

Wider implications of the findings: The measurement of ANA might not be necessary for the screening of patients with RPL who have no features of collagen disease.

Trial registration number: not applicable

P-382 Pre-selected for an award: Association of extended culture to blastocyst and gestational trophoblastic disease risk following IVF/ICSI assisted reproduction cycles: An analysis of large UK National database

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Study question: Is there any association between stage of embryo at transfer based on extended in vitro culture and gestational trophoblastic disease risk during assisted reproduction?

Summary answer: No significant association between stages of embryo transfer from zygote stage to blastocyst stage was found after analysing 540376 cycles of IVF and ICSI.

What is known already: Gestational trophoblastic disease (GTD), commonly referred to as molar pregnancy, results from abnormal proliferation of the trophoblast with a reported incidence of ~1 in 700 in the UK. Despite technological advances such as ICSI, PGT and selection of normally fertilised (2PN) embryos, there are reported cases of GTD following assisted reproduction. Blastocyst transfer is associated with higher pregnancy and live birth rates but evidence is lacking whether extended embryo culture to blastocyst stage influences implantation of an abnormal embryo or abnormal trophoblastic proliferation leading to GTD.

Study design, size, duration: A retrospective study was carried out using Human Fertilisation and Embryology Authority (HFEA) anonymised register data from 1999 to 2016. HFEA holds the longest running register for fertility treatment data in the world and is the national database for fertility treatment data in UK. A total of 540376 fresh IVF or ICSI assisted reproduction cycles were analysed.

Participants/materials, setting, methods: There were 1033588 treatment cycles during the study period but only 540376 cycles met the inclusion criteria of fresh IVF or ICSI. Cycles with incomplete data, frozen embryo transfers, donor treatment or surrogacy were excluded. A subgroup analysis of those with primary subfertility was performed after excluding subjects with secondary infertility in order to exclude an effect of a previous molar pregnancy. Multivariate regression analysis was used to adjust for possible confounders.

Main results and the role of chance: 78 molar pregnancies were reported in the original sample giving a prevalence of 4/10000 live births (78/228461), much lower than the prevalence given with natural pregnancies. Prevalence of molar pregnancy amongst the study population after meeting exclusion criteria was 4 /10000 livebirths (53/156683). Incidence of molar pregnancy was not statistically different between treatment type (0.0001 vs 0.00009).

Significantly higher incidence of GTD was seen in the 40 to 42 age category compared to 18-34 category(OR 1.86(95% CI 8.7-3.75)),in par with known higher GTD risk in women above 40 in the general population. Of interesting note, although the incidence of molar pregnancy was significantly lower in women undergoing assisted reproduction increased risk with advancing age is not totally eliminated with treatment. There was no significant association between the occurrence of molar pregnancy with the type and cause for infertility and number of embryos transferred.

Crude (1.06 (95% CI 0.852-1.31)) and adjusted odds ratios (1.07 (95% CI (0.857-1.32)) did not show any association between day of embryo transfer and molar pregnancy even after adjusting for age and excluding secondary infertility. Selection of blastocyst stage embryo after extended culture did not alter the likelihood of having a GTD compared to cleavage stage embryo.

Limitations, reasons for caution: The retrospective analysis of anonymised HFEA data limited adjustments for confounders such as smoking, previous history of GTD, ethnicity etc that predispose to GTD. Caution needs to be exercised for under-reporting of GTD to HFEA and lack of information on type of GTD identified.

Wider implications of the findings: Though GTD cannot be prevented by IVF/ICSI, the incidence is significantly low and extended culture is not associated with higher risk of abnormal trophoblastic proliferation or GTD occurrence with IVF/ ICSI treatment. These findings would aid informed implications counselling and reassurance of patients during assisted reproduction treatments.

Trial registration number: not applicable

P-388 Pre-selected for an award: Endometrial extracellular vesicles from recurrent implantation failure patients inhibited embryonic growth and implantation via miR-6131/PAK2 pathway

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Study question: Could endometrial extracellular vesicles from recurrent implantation failure patients (RIF-EVs) attenuate the growth and implantation potentials of embryos and what are the mechanisms?

Summary answer: RIF-EVs inhibited embryonic growth and decreased the trophoblast functions via miR-6131/PAK2 pathway.

What is known already: Recurrent implantation failure (RIF) is characterized by repeated embryo transfers without pregnancy. To date, the etiology of RIF remains poorly understood. Recent evidence indicated that extracellular vesicles (EVs) secreted by endometrial cells, played a crucial role in the implantation by regulating the development and implantation of embryos.

Study design, size, duration: Endometrial cells isolated from endometrial tissues of RIF patients (n=25) and fertile women (n=16) were cultured and modulated via hormones. Endometrial EVs from RIF patients (RIF-EVs) or fertile women (FER-EVs) were isolated from the conditioned medium. The influence of EVs on embryonic development and implantation was investigated by co-culture models of EVs and 2-cell murine embryos or HTR8/SVneo cells, respectively. High-through put sequencing was performed to identify the miRNA profile in the RIF-EVs.

Participants/materials, setting, methods: RIF-EVs and FER-EVs were characterized using western blotting, nanoparticle tracking analysis, and transmission electron microscopy. After co-culture with EVs, embryonic blastocyst rate and hatching rate were calculated. Besides, the proliferation, migration, and invasion of EV-treated trophoblast cells were evaluated by CCK-8, wound healing, and transwell invasion assays. miRNA expression profiles were compared between RIF-EVs and FER-EVs, and the regulatory role of significantly upregulated miR-6131 in RIF-EVs was investigated in the trophoblast cells.

Main results and the role of chance: RIF-EVs and FER-EVs are round bilayer vesicles, ranging mainly at 100 nm and enriched in TSG101, Alix, and CD9. Both RIF-EVs and FER-EVs entered embryonic or trophoblast cytoplasm. The blastocyst rate in the RIF-EV groups was significantly decreased compared to that in the FER-EV groups, at concentrations of 5, 10, and 20 μg/ml. The hatching rate was decreased significantly in embryos treated with 10 or 20 μg/ml RIF-EVs compared to those treated with FER-EVs at the same concentration ($p<0.05$). The proliferation, migration, and invasion of trophoblasts were significantly decreased in the RIF-EV group at 20 μg/mL. A total of 11 differently expressed (fold change >2 and $p< 0.05$) miRNAs were found in the RIF-EVs, and two of them were validated in a larger set of EV samples using RT-PCR. The most significantly different miRNA, 6131, was increased in the RIF-EV-treated HTR8/SVneo cells. The up-regulation of miR-6131 inhibited the growth and invasion of HTR8/SVneo. Bioinformatics coupled with luciferase and western blot assays revealed that PAK2 is a direct target of miR-6131, and the overexpression of PAK2 can rescue the phenotype changes induced by miR-6131 overexpression.

Limitations, reasons for caution: Our study indicated miRNA in the RIF-EVs dysregulating the growth and function of embryonic cells. However, EVs contained a wide spectrum of bioactive molecules, including proteins, mRNAs, and DNA, which may play an important role in the implantation. Further studies are required to investigate the mechanisms.
Wider implications of the findings: This work indicates an important role of EVs from women with RIF in embryonic implantation, potentially providing a novel insight to understand the pathophysiology of RIF.
Trial registration number: not applicable

P-420 Pre-selected for an award: Uncomplicated oocyte donation pregnancies display elevated CD163 positive type 2 macrophage load in the decidua, which is associated with fetal-maternal HLA class II mismatches

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Study question: Do quantity and composition of decidual macrophages differ between uncomplicated oocyte donation (OD) pregnancies and non-OD in vitro fertilization (IVF) pregnancies?
Summary answer: OD placentas show higher decidual CD163 positive fraction within the total macrophage population compared to non-OD IVF placentas.
What is known already: The embryo of an OD pregnancy is completely allogeneic to the mother, which may lead to a bigger challenge for the maternal immune system to tolerize the fetus compared to autologous pregnancies. Placental macrophages may be essential in maintaining a healthy pregnancy. Macrophages can be classified into different categories based on phenotype and characteristics, in which type 2 macrophages are thought to exhibit immune suppressive activity.
Study design, size, duration: This retrospective case-control study included patients who delivered in the Leiden University Medical Center between January 1st 2006 and July 1st 2016. A total of 42 pregnancies were enrolled in this study, conceived by uncomplicated singleton OD pregnancies (n=25) or non-OD IVF pregnancies (n=17). Medical records were reviewed and clinical data were collected. Placental tissue samples were collected for immunohistochemical staining and blood samples were collected for HLA typing.
Participants/materials, setting, methods: Placentas were collected and immunohistochemically stained for CD14 (pan-macrophage marker) and CD163 (type 2 macrophage marker). The extent of staining was quantitated by digital image analysis software. To assess mismatching, maternal and fetal DNA was typed for HLA-A, -B, C, -DRB1, and -DQB1.
Main results and the role of chance: A significantly lower percentage of CD14 positive staining was observed in the decidua basalis of OD pregnancies compared to non-OD IVF pregnancies (p=0.030). Consequently, the CD163/CD14 ratio in OD group was higher than in non-OD IVF group (p=0.243). In the parietalis, OD pregnancies demonstrated a significantly higher percentage of CD163+ staining (p=0.040) and a significantly higher CD163/CD14 ratio (p=0.032) compared to non-OD IVF group. The reproducibility of this quantitative analysis was found to be high. OD group was separated into a syngeneic group (number of mismatches lower than half of the antigens per HLA locus) and an allogeneic group (number of mismatches higher than half of the antigens per HLA locus). Significant differences of CD163+ and CD163/CD14 ratio were found in the decidua parietalis when comparing the HLA-classII-allogeneic OD group with the non-OD IVF group (p=0.047). This difference was not found for the HLA-class-II-syngeneic OD group.
Limitations, reasons for caution: Our study only focused on decidua basalis and parietalis, no other locations in the placentas. Larger sample size might be needed to verify the association between macrophages and HLA mismatches.
Wider implications of the findings: To our knowledge, this study is the first to quantify a higher CD163 positive M2 macrophages load within the total decidual macrophages of uncomplicated OD pregnancy compared to non-OD IVF pregnancies.
Trial registration number: not applicable

POSTER DISCUSSION

SESSION 61: ENDOMETRIOSIS, ENDOMETRIUM AND FALLOPIAN TUBE POSTER DISCUSSIONS

30 June 2021 Stream 4 15:15 - 16:30

P-295 Does endometriosis affect oocyte quality? An analysis of 13 627 donor oocyte recipient and autologous IVF cycles.

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Study question: Does endometriosis affect live birth following donor oocyte recipient versus autologous in vitro fertilisation (IVF) cycle.
Summary answer: There was no significant difference in the live birth rate (LBR) in women with endometriosis undergoing donor oocyte recipient versus autologous IVF cycle.
What is known already: For infertile women with endometriosis, IVF is often considered as a treatment option. Lower implantation and pregnancy rates have been observed following IVF in women with endometriosis when compared to tubal factor infertility. It has been debated that lower pregnancy rates following IVF in endometriosis is due to both oocyte quality and the endometrium. To delineate whether endometriosis affects oocyte quality or the endometrium, we planned a study using donor oocyte recipient model where the recipient were women with endometriosis. We compared the LBR after oocyte recipient cycle with autologous IVF in women with endometriosis
Study design, size, duration: We obtained anonymised dataset of all the IVF cycles performed in the UK since 19991 from the Human Fertilization and Embryology Authority (HFEA). Data from 1996 to 2016 comprising a total of 13 627 donor oocyte recipient and autologous IVF cycles with endometriosis and no other cause of infertility were analysed.
Participants/materials, setting, methods: Data on all women with endometriosis undergoing fresh or frozen IVF treatment cycles were analysed to compare the LBR between donor oocyte recipient and autologous treatment cycles. Logistic regression analysis was performed adjusting for number of previous IVF cycles, previous live birth, period of treatment, day of embryo transfer, number of embryo transferred, fresh and frozen cycle.
Main results and the role of chance: There was no significant difference in the LBR in women with endometriosis undergoing donor oocyte recipient fresh cycles compared to women undergoing fresh autologous IVF cycles (31.6% vs. 31.0%; odds ratio, OR 1.03, 99% CI 0.79 – 1.35). After adjusting for confounders listed above, there was no significant difference in LBR in women undergoing donor oocyte recipient fresh cycles versus fresh autologous ART cycles (aOR 1.06, 99% CI 0.79 – 1.42).

There was no significant difference in the LBR in women with endometriosis undergoing frozen donor oocyte recipient cycles compared to women undergoing autologous frozen embryo transfer cycles (19.6% vs. 24.0%; OR 0.77, 99% CI 0.47 - 1.25). After adjusting for potential confounders, there was no significant difference in the LBR in women undergoing frozen donor oocyte recipient cycles compared with autologous frozen embryo transfer cycles (aOR 0.84, 99% CI 0.50 - 1.41).
Limitations, reasons for caution: Although the analysis was adjusted for several potential confounders, there was no information on classification of endometriosis to allow adjustment.
Wider implications of the findings: The current study design does not indicate endometriosis has an impact on oocyte quality given that the outcomes in donor oocyte recipient cycles are comparable with autologous IVF cycles. These findings need to be further studied and validated.
Trial registration number: Not applicable

P-296 Examining the link between environmental toxin exposure and uterine leiomyoma: a systematic review

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Study question: Is there an association between exposure to certain environmental toxins and the prevalence of uterine leiomyoma in women?
Summary answer: Some evidence was obtained to suggest an association between phthalate esters, bisphenol A, heavy metals, persistent organic pollutants and the prevalence of uterine fibroids.
What is known already: Environmental toxins are naturally occurring, or human made chemicals that can act as endocrine disrupting chemicals (EDCs) by binding and activating estrogen receptors in the body. Uterine fibroids, often called leiomyoma are non-cancerous growths occurring in the uterus. Though often asymptomatic, they can cause pain, infertility, pregnancy complications and are a leading cause for hysterectomy. The aetiology of leiomyoma is not fully understood but both estrogen and progesterone have been implicated in their growth. We aimed to investigate the epidemiological evidence for the association between EDCs and the prevalence of fibroids.
Study design, size, duration: We undertook a systematic review and in keeping with PRISMA guidelines, a structured search of Medline, Embase, Scopus, and Web of Science was conducted (to October 2020). Case-control, cross-sectional, cohort and experimental studies were included.
Participants/materials, setting, methods: The included studies analyzed the association between one or more toxins and the occurrence, or growth of leiomyoma in humans, including human cell lines. The types of toxins, patient characteristics, association and outcome, body concentration of toxin and confounding variables were extracted and analyzed. Quality assessment was performed using the Newcastle-Ottawa Scale.
Main results and the role of chance: In total, 34 studies were included. The majority (76%) of studies revealed a significant association between the exposure studied and the prevalence of uterine leiomyoma. In examining body burden in cases vs controls, phthalate esters showed an association with increased odds of uterine leiomyoma, except in one case where a negative association was observed. In vitro experimental studies examining the effect of alkyl-phenols such as bisphenol A (BPA), octylphenol (OP) and nonylphenol (NP) demonstrated that these environmental estrogens can act to promote the proliferation of leiomyoma cells through a number of mechanisms, typically including the estrogen receptor alpha (ERa) signalling pathway. There were conflicting results for the association between alkyl-phenols and fibroids in case-control studies. A positive association between cadmium was demonstrated in only two studies. There were conflicting results for the association between lead, mercury, arsenic and uterine fibroids. Several metabolites of organophosphate esters, alternative plasticizers, and persistent organic pollutants were associated with an increased risk of uterine fibroids.
Limitations, reasons for caution: Separating these exposures from the multiple other factors that could affect the outcome of leiomyoma is challenging, but an important issue for future research.
Wider implications of the findings: The link between some environmental toxins and uterine fibroids discussed is in agreement with previous literature. However, our review provides a more in depth analysis on specific dosage effects, odds ratios, and potential gene mechanisms of the exposures. This information could contribute to more accurate preventative measures.
Trial registration number: not applicable

P-307 Fatty acid degradation during in vitro decidualization of human endometrial stromal cells

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Study question: Is the activity of the β-oxidation pathway, involved in the degradation of fatty acids, modified during *in vitro* decidualization of human endometrial stromal cells (HESC)?
Summary answer: The level of expression of fatty acid´s transporters suggests that the activity of the mitochondrial β-oxidation pathway is increased during *in vitro* decidualization of HESC.
What is known already: The differentiation of endometrial stromal cells (ESC), named decidualization, is essential for the proper formation of the materno-fetal interphase. One important feature of decidualization is the increased glucose consumption. In the endometrium, glucose is incorporated into ESC by glucose-transporters (GLUT). Fatty acids are another important energy source in living cells. Fatty acids are transported into mitochondria by the carnitine-palmitoyl-transferases 1 and 2 (CPT1 and 2) and are degraded there through the β-oxidation pathway. It has been described that the inhibition of CPT1 affects ESC decidualization. However, it is unknown whether the turn-over of fatty acids degradation is modified during decidualization.
Study design, size, duration: This study was performed using primary HESC. Endometrial biopsies (mid-late proliferative-phase) were obtained from healthy-regularly-cycling women (33.6±2.2 years-old) after written informed consent was obtained (protocol approved by Ethics committee no. S-239/2005). HESC were decidualized (D) *in vitro* with a decidualization-cocktail (containing: medroxyprogesterone acetate, estradiol and 8-Bromo-cyclic adenosine monophosphate) for 6 days. Non-decidualized (ND) controls were treated with vehicle solutions. Cell-culture supernatant and cell extracts were collected for the evaluation of protein/gene expression and metabolite content.
Participants/materials, setting, methods: Decidualization was evaluated by measuring prolactin (PRL) protein levels in cell-culture supernatant (mU/l). Changes in mRNA expression levels of *GLUT1*, *CPT1A* and *CPT2* were evaluated by real-time polymerase chain reaction (RT-PCR). Analysis was performed by the ΔΔCt method (internal control: *RPLP0*) (fold change -FC- in D compared to ND cells). Contents of acylcarnitines were evaluated by Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS) (nmol/mg of total protein). N=5, mean±SEM. Paired Student's t-test was used for statistical analysis.
Main results and the role of chance: PRL protein levels in cell-culture supernatant were significative increased in HESC treated with the decidualization-cocktail compared to ND cells (ND 16.80±0.73 mU/l; D 684.20±219.80 mU/l, *p<0.05). This result confirmed the decidualized state of HESC upon *in vitro* treatment with the decidualization-cocktail. Additionally, the mRNA expression level of *GLUT1* was highly upregulated in D compared to ND cells (FC 10.02±2.90, ***p<0.001), consistent with the increase in glucose consumption characteristic of decidualization. Once confirmed the decidualized state of HESC, the mRNA expression levels of *CPTA1* and *CPT2* were evaluated. The mRNA expression levels of both fatty acid´s transporters were upregulated in D compared to ND cells (*CPTA1*: FC 1.84±0.44, **p<0.01; *CPT2*: FC 2.04±0.49, **p<0.01). Finally, the content levels of different acylcarnitines, intermediate metabolites of the β-oxidation degradation of fatty acids, were evaluated. The concentrations of acetyl- (C2) and butyryl- (C4) acylcarnites were decreased in D compared to ND cells [(C2: ND 1.37±0.10 nmol/mg of total protein; D 1.06±0.20 nmol/mg of total protein, *p<0.05), (C4: ND 0.03±0.01 nmol/mg of total protein; D 0.01±0.00 nmol/mg of total protein, *p<0.05)]. The content levels of other intermediate acylcarnitines measured from cell extracts had no differences between D and ND cells (p>0,05).
Limitations, reasons for caution: This study was performed *in vitro* using primary HESC treated with a decidualization-cocktail. The interconnection of different metabolic pathways within a living cell is very complex. Further studies are necessary to define whether the different intermediate metabolites of the mitochondrial β-oxidation pathway are being used by related-metabolic pathways during decidualization.
Wider implications of the findings: The regulation of the energy metabolism and its interconnection with other important intra-cellular metabolic pathways is of great importance for cellular function. Our results contribute to highlight the importance of the regulation of fatty acids degradation during decidualization. Further insights into HESC metabolism could facilitate the improvement of women´s health.
Trial registration number: not applicable

P-321 The impact of endometrioma and ovarian cystectomy in patients with major indications for IVF/ICSI with endometriosis

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Study question: Does presence of endometrioma has worse IVF/ICSI outcome than endometriosis per se? What about the impact of cystectomy of endometrioma on IVF/ICSI outcomes?
Summary answer: IVF/ICSI outcome of patients with endometrioma is comparable than with endometriosis. Cystectomy for endometrioma did not alter IVF/ICSI outcomes if ovarian reserve is comparable.
What is known already: Previous studies revealed women with endometrioma undergoing IVF/ICSI had similar reproductive outcomes compared with those without. Most of the comparisons are between women with endometrioma and women without endometriosis. However, endometrioma per se, different from endometriosis may have specific impact on IVF/ICSI outcomes. There is now molecular, histological and morphological evidence to suggest endometrioma is detrimental to the ovaries. Studies comparing IVF/ICSI outcomes between women with endometrioma and women with endometriosis are few.

Cystectomy of endometrioma may worse ovarian reserve, and subsequently adversely affect IVF/ICSI outcomes. But there are possible complications associated with the persistence of endometrioma during IVF/ICSI.
Study design, size, duration: Retrospective analysis of 2153 IVF/ICSI cases during Jan/01/2014 to Dec/31/2018 in VGHTC. We included women who received ART due to endometriosis(n=208). Exclusion criteria including patients >40 years-old, simulation day < 5 days, severe male factor, uterine factor (including adenomyosis) and immunological factors. Patients whose embryos were not completely transferred back or who received embryo transfer from different OPU cycles are excluded. We followed up these patients till 2020/6. The primary outcome is cumulative LBR
Participants/materials, setting, methods: For first analysis, we divided 208 cases to patients with endometrioma during IVF/ICSI(n=89), and patients only diagnosed of endometriosis (n=119). Second analysis on the effect of cystectomy of endometrioma on IVF/ICSI outcomes. Patients with endometrioma (n=89) during IVF/ICSI were further divided to patients with primary endometrioma (n=70) and patients with recurrent endometrioma (n=19, ever received cystectomy for endometrioma). Another group is patients without endometrioma during IVF/ICSI, but ever received cystectomy before (n=40)
Main results and the role of chance: For the first analysis, age, BMI and AMH were comparable in endometrioma (n=89) and endometriosis group(n=119). The usage gonadotropin dose was significantly higher in the endometrioma group (FSH 3619IU vs 3471IU, p=0.001. LH 1224 IU vs 941 IU, p=0.009). The Blastocyst formation rate is lower in the endometrioma group (49.4% vs. 57.7% p=0.005). The OPU number, LBR and cumulative LBR were comparable in both groups (10.3 vs 12.4 p=0.131, 33.3% vs 37%, p=0.687, 49.4% vs 60.5%, endometrioma vs endometriosis). For the second analysis, when comparing cystectomy before IVF/ICSI group with primary endometrioma group, cystectomy group were younger (32.8 vs 34.8 p=0.006). AMH level were comparable. The BC formation rate was significantly higher in the cystectomy group (61.5% vs 50.4% 0= 0.007). The LBR and cumulative LBR were comparable in both groups (43.5% vs 28.1%, 60% vs 48% in cystectomy vs primary endometrioma group). As for the recurrent endometrioma group, the age and AMH level were comparable with cystectomy group, but the usage gonadotropin dose was significantly higher than other two groups. The BC formation rate was also lower than cystectomy group (47.8% vs 61.5% p=0.042). The LBR and cumulative LBR were comparable with other two groups (55.6%, 57.9%).
Limitations, reasons for caution: This is a retrospective study, and the sample size is limit. We did not analysis the size of endometrioma nor the unilateral or bilateral endometrioma.
Wider implications of the findings: Cystectomy for endometrioma must be carefully selected since it did not alter IVF/ICSI outcome only if the ovarian reserve is not affected. Recurrent endometriomas do not have a worse impact on IVF/ICSI outcome than primary endometrioma. If there is recurrent endometrioma, IVF/ICSI may be the first priority.
Trial registration number: not applicable

P-322 Addressing progesterone and cAMP signalling pathways for decidualization induction of endometrial stromal cells of patients with endometriosis

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Study question: Which compounds/compound combinations are most effective in decidualization induction of endometrial stromal cells (ESCs) of patients with and without endometriosis?
Summary answer: Combination of compounds addressing different steps in the signalling cascade of decidualization induce decidualization more effectively than application of the individual compounds alone.
What is known already: Decidualization is the monthly recurring differentiation process of the ESCs in preparation for embryo implantation in human. Undifferentiated ESCs reveal an increased potential to proliferate and invade after retrograde menstruation. This may lead to the formation of ectopic lesions and the manifestation of the chronic gynaecological disease of endometriosis due to an impairment of the decidualization process.
Study design, size, duration: Compounds and compound combinations addressing the progesterone receptor- or the cAMP-mediated pathway were evaluated with regard to their own and their synergistic potential to induce decidualization of ESCs from women with (n=10) and without (n=10) endometriosis during a 6-day treatment.
Participants/materials, setting, methods: Human primary ESCs were isolated via enzymatic-mechanic digestion from eutopic endometrium from women with and without endometriosis and treated for 6 days in vitro with different progestins (progesterone, medoxyprogesterone acetate (MPA)), 8-Br-cAMP, forskolin, or phosphodiesterase (PDE)-inhibitor (Rolipram) alone or in combination. The degree of decidualization induction was quantified by morphological, biochemical (prolactin) and molecular (HAND2, FOXO1) parameters by means of ELISA, flow cytometric analysis, Realtime PCR and Western blot analysis.
Main results and the role of chance: After 6 days of treatment, decidualization was induced by forskolin as well as by 8-Br-cAMP whereas progestins or PDE alone hardly induced prolactin secretion by ESCs as a marker of decidualization. A change of morphology from undifferentiated fibroblast-like cells to rounded cells could be observed in parallel with the secretion of prolactin. Forskolin and 8-Br-cAMP-induced decidualization was significantly enhanced by MPA but not by progesterone. These effects were similar in ESCs from women with and without endometriosis. Moreover, forskolin-induced decidualization was significantly enhanced by simultaneous application of PDE. Interestingly, this effect was higher in cells of patients with endometriosis. An induction of decidualization in ESCs was associated with a parallel increase of the process-associated transcription factors HAND2 and FOXO1. This rise of transcription was markedly increased in combination with MPA but not with progesterone.
Limitations, reasons for caution: Endometrial tissue was obtained from women undergoing infertility treatment and thus may differ from the endometrium of fertile women. Results obtained from primary cells in vitro may not cover the in vivo situation in all respects.
Wider implications of the findings: The results of this study provide baseline data for the development of a possible therapeutical approach to induce decidualization as a treatment option for endometriosis. Further research is required to determine the effectiveness of the in vitro tested compound combinations in an in vivo model.
Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 62: MALE AND FEMALE FERTILITY PRESERVATION: INDICATIONS AND OUTCOME

30 June 2021 Stream 1 17:00 - 18:00

O-177 Long-term follow up to assess criteria for ovarian tissue cryopreservation for fertility preservation in young women and girls with cancer

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Study question: Do the Edinburgh selection criteria correctly identify females, diagnosed with cancer under 18 years old, at high risk of future premature ovarian insufficiency (POI)?

Summary answer: Patient assessment using these criteria accurately identifies those at risk of POI. Ovarian tissue cryopreservation with future transplantation can be offered, providing future fertility options.

What is known already: Cancer treatments can be gonadotoxic and future fertility and reproductive health should be considered at the time of diagnosis and treatment planning. Correct identification of patients at high risk allows appropriate discussion of fertility preservation with ovarian tissue cryopreservation (OTC) and future transplantation. The Edinburgh selection criteria have been proposed as a tool to identify those patients at high risk.

However, the surgical procedure is not without risk and reproductive outcomes remain uncertain in girls. Therefore, long-term follow up of reproductive function is crucial to ensure that this treatment strategy is offered appropriately.

Study design, size, duration: All females diagnosed with cancer less than 18 years old, in South East Scotland, between 01/01/96 and 30/10/20 were included. They were assessed using the Edinburgh selection criteria and offered OTC, if appropriate. Ongoing long-term follow up of reproductive outcomes has been undertaken for the whole patient cohort to detect those who develop POI.

Participants/materials, setting, methods: A total of 639 eligible patients were identified from the Cancer registry and their electronic records reviewed. Reproductive function was assessed by the presence of menstruation, pregnancy, hormonal measurements, evidence of puberty or diagnosis of POI. Patients on hormonal contraception (other than for the treatment of POI) were considered unsuitable for analysis.

Data were analysed using the Kaplan Meier method, with POI as the event, and the Cox proportional hazards model to calculate hazard ratios.

Main results and the role of chance: Of the 639 patients diagnosed with cancer during the study period, those deceased before age 12 years old (n=73) or under 12 years old (n=134) at the date of analysis were excluded; also excluding those on hormonal contraception (n=9) gave a study population of 423.

Data were analysed including those with unknown reproductive outcomes (n=143), assuming they did not have POI. A subgroup analysis excluding these patients was also performed.

Mean age at diagnosis and analysis was 8.8 years and 22.5 years respectively.

OTC was offered to 37 patients, 26 of whom underwent the procedure. Nine patients developed POI (24.3%). Of the 386 not offered OTC, 11 developed POI (2.85%). The hazard ratio for developing POI was 8.8 (CI 3.6-21).

Excluding the patients with unknown outcomes (n=143) left a study population of 280. Within this group, 9 of 29 offered OTC developed POI (31.0%) versus 11 of 251 not offered OTC (4.4%); hazard ratio 8.2 (CI 3.4-20).

In the group offered OTC, all cases of POI developed after the primary treatment. In those not offered OTC, POI developed after secondary treatment for disease relapse in 5 patients (45.5%).

Limitations, reasons for caution: A significant number of patients had unknown reproductive outcomes; this is likely to reflect a lack of recording of normal menstrual function in oncology/haematology clinics but may have biased the analysis. The duration of follow up is also short for some patients, highlighting the need for further follow up.

Wider implications of the findings: The overall prevalence of POI after childhood cancer is low, but the Edinburgh selection criteria are a robust tool for selecting those at high risk at the time of diagnosis, who can be offered OTC. However, many patients had incomplete information on current reproductive status, which should be assessed routinely.

Trial registration number: N/A

O-178 Reproductive and endocrine outcomes after fresh and frozen-thawed ovarian tissue transplantation based on age and anti-cancer therapy: A systematic review and individual patient data meta-analysis

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Study question: Do reproductive and endocrine outcomes from fresh and frozen-thawed ovarian transplants differ based on age and anti-cancer therapy before cryopreservation?

Summary answer: There was a significant difference in reproductive outcomes of women who have their tissue cryopreserved before or at the age of 35 years.

What is known already: Ovarian tissue cryopreservation (OTC) and transplantation is emerging as a new fertility preservation method. Despite being available for two decades, there is a marked variation in the delivery of this procedure worldwide. Most of the data are based on case reports from specialised centres with expertise in providing this procedure, but there are many unreported cases. Through this review, we aim to collate reproductive and endocrine outcomes from ovarian tissue transplantation. In particular the outcomes in women based on age at cryopreservation and whether they had anti-cancer therapy before cryopreservation were explored.

Study design, size, duration: This study was a systematic review and individual participant level meta-analysis to synthesize the existing evidence on the use of fresh and cryopreserved ovarian tissue transplantation. The review protocol was registered with PROSPERO (CRD42018115233) in November 2018 and the review was concluded in December 2020, including 87 studies (768 women).

Participants/materials, setting, methods: Literature search was performed using MEDLINE, EMBASE, CINAHL and Cochrane Central Register of Controlled Trials from inception to October 2020. After screening 20,566 abstracts, 87 studies (768 women) were included in the review. Patient-level data was extracted for 388 women and study-level data for 380 women. Authors were also contacted for data if relevant outcomes were not reported in published manuscripts. Meta-analysis was performed using inverse-variance weighting to calculate summary estimates using a fixed-effects model.

Main results and the role of chance: Age at cryopreservation was provided for 319 out of 388 (82%) women at participant level data. Of these, 283 (88.7%) had ovarian tissue retrieved at ≤35 years of age. A subgroup of four studies that reported data on participants age at cryopreservation and transplantation were included in meta-analysis. Pregnancy rates were higher in participants at ≤35 years of age at cryopreservation, with results being statistically significant (OR, 0.35; 95% CI: 0.13 to 0.92; z = 2.13; P 0.03, I^2 = 0%). Return of hormonal function shown as a decrease in FSH (IU/L) was also lower in this group (MD, 4.38; 95% CI: -4.29 to 13.05; z = 0.99; P 0.32, I^2 = 0%. Whether a participant had received chemotherapy before cryopreservation was explicitly reported in 122 out of 388 (31%) participants and 56 of them (46%) had received anti-cancer treatment before OTC. Thirty-five pregnancies and twenty-four live births were reported in these women. A further meta-analysis from 5 studies showed that although the results were not statistically significant for return of endocrine function, a decrease in FSH, an increase in oestrogen and increased pregnancy rates were noted in participants who did not receive anti-cancer therapy before cryopreservation.

Limitations, reasons for caution: Although we gathered 768 cases of ovarian transplants published in the literature, most were case reports and therefore not included in the meta-analysis. Of the studies included in the meta-analysis, information such as age and anti-cancer therapy were not always provided for individual participants but as an aggregate.

Wider implications of the findings: There was no difference in reproductive and endocrine outcomes for anti-cancer therapy before OTC. Previous

chemotherapy alone should therefore not be a deterrent in offering young girls and women OTC. Furthermore, the ideal age to achieve higher pregnancy and live birth rates from OTC is less than 35 years.
Trial registration number: PROSPERO (CRD42018115233)

O-179 Safety of ovarian tissue cryopreservation and transplantation in patients with central nervous system cancers

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Study question: Is cryopreserved ovarian tissue transplantation safe in patients with central nervous system (CNS) tumors?
Summary answer: Cancer cell contamination was not detected in any ovarian samples from patients with CNS tumors by histological analysis, immunohistochemistry, molecular biology or long-term xenotransplantation.
What is known already: Frequently encountered CNS cancers in childhood include astrocytoma, medulloblastoma, ependymoma, glioblastoma and germinoma. CNS tumors have the capacity for extraneural metastases in 0.5-18% of cases. There are two publications reporting metastases to patients' ovaries from medulloblastoma.
Study design, size, duration: Prospective experimental study conducted in an academic gynecology research laboratory using frozen-thawed ovarian tissue from 20 patients suffering from 6 types of CNS tumors, including the most common forms mentioned above and primitive neuroectodermal tumors (PNET). Five-month xenotransplantation was performed to severe combined immunodeficient (SCID) mice.
Participants/materials, setting, methods: Cryopreserved ovarian tissue from 20 patients with CNS cancers was thawed and analyzed for minimal disseminated disease and long-term xenografting to immunodeficient mice. The presence of malignant cells was assessed in both cryopreserved and xenografted ovarian tissue using histological analysis, immunohistochemistry for disease-specific markers (neuron-specific enolase [NSE] and glial fibrillary acidic protein [GFAP]) and reverse transcription droplet digital polymerase chain reaction (RT-ddPCR) for quantification of GFAP gene amplification.
Main results and the role of chance: No malignant cells were detected in frozen-thawed ovarian tissue from any of the patients by histology, immunolabeling for NSE and GFAP, RT-ddPCR for detection of GFAP gene amplification or xenotransplantation to SCID mice. One patient successfully underwent frozen-thawed ovarian tissue transplantation, resulting in the birth of 3 healthy children, but suffered a recurrence of her PNET 6 years after reimplantation and sadly died. Scrupulous analysis of her remaining frozen tissue showed no infiltration by malignant cells, neither after thawing nor long-term xenotransplantation. No relationship was ever established between the patient's relapsed cancer and reintroduction of her cryopreserved ovarian tissue. The risk of reseeding cancer cells when transplanting ovarian tissue in patients with CNS cancers can therefore be considered low.
Limitations, reasons for caution: The risk of ovarian metastases cannot be completely ruled out for any type of tumor because we cannot analyze the actual fragments that will be reimplanted.
Wider implications of the findings: Our results indicate that the risk of disseminated disease in ovarian tissue from CNS patients is minimal. This is useful information for doctors when counseling women looking to undergo ovarian tissue transplantation.
Trial registration number: Not applicable

O-180 Oocyte vitrification for fertility preservation does not delay the initiation of neoadjuvant chemotherapy for breast cancer.

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Study question: Does oocyte vitrification for fertility preservation (FP) delay the initiation of neoadjuvant chemotherapy for breast cancer?
Summary answer: The indication of neoadjuvant chemotherapy for breast cancer should not be considered as an impediment to urgent oocyte vitrification for FP.
What is known already: FP is considered as one of the most important issues to address for young breast cancer patients. Cryopreservation of oocytes or embryos may be considered after controlled ovarian hyperstimulation (COH) or in vitro maturation (IVM). Pregnancies have been reported after reutilization of oocytes frozen following both procedures. Although oocyte competence is better after COH, this strategy requires on average 13 days to be achieved. In addition, the safety of ovarian stimulation before tumor removal is currently not formally established. In case of neoadjuvant chemotherapy, the risk-benefit balance of COH is not well known.
Study design, size, duration: Retrospective cohort study including all breast cancer patients eligible for oocyte vitrification following COH or IVM before initiation of neoadjuvant chemotherapy between January 2016 and December 2020.
Participants/materials, setting, methods: Inclusion criteria were: female patients with confirmed non metastatic breast cancer, 18 to 40 years of age, with indication of neoadjuvant chemotherapy, who have had oocyte retrieval for FP after COH or IVM +/- cryopreservation of ovarian tissue. Various time-points related to cancer diagnosis, FP or chemotherapy were obtained from medical record review.
Main results and the role of chance: A total of 198 patients with confirmed breast cancer who had oocyte retrieval following COH (n=57) or IVM +/- cryopreservation of ovarian tissue (n=141) for FP prior to neoadjuvant chemotherapy were included. Although women in IVM group were significantly younger as compared to patients who underwent COH (31.7 ± 4.2 *vs.* 33.3 ± 4.0 years, *p=0.019*), ovarian reserve parameters, BMI and cancer stage did not differ between the two groups. Overall, the average time from cancer diagnosis to chemotherapy start was similar between patients having undergone COH or IVM before oocyte vitrification (37.3 ± 13.8 *vs.* 36.9 ±13.5 days in COH and IVM groups respectively, *p=0.857*).
Limitations, reasons for caution: The time from referral to FP consultation may have influenced the type of FP. In addition, the retrospective nature of the present analysis may constitute a limitation. Moreover, the efficiency and security of the different FP strategies used has not been analysed.
Wider implications of the findings: Oocyte vitrification following COH or IVM was not associated with delayed breast cancer treatment in the neoadjuvant setting, so long as there was a prompt FP referral. Young patients undergoing neoadjuvant chemotherapy should be informed of these findings to avoid unnecessary anxiety due to concern for delays.
Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 63: EFFECTIVE EMBRYO TRANSFER, PREGNANCY RISKS AND MATERNAL IMPACT ON ART OUTCOME

30 June 2021 Stream 2 17:00 - 18:00

O-181 4D ultrasound guided embryo transfers statistically improve live birth rates - A randomised controlled trial

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Study question: Does the use of 4D ultrasound to guide embryo transfers improve live birth rates in comparison to the clinical touch technique?
Summary answer: 4D ultrasound guided embryo transfers (4DUS) result in significantly higher live birth rates (LBR) in comparison to those performed using the clinical touch technique (CTT)(41%vs28%).
What is known already: A previous Cochrane review showed ultrasound guided embryo transfers (ET) improve pregnancy outcomes in comparison to CTT; however there was a large degree of heterogeneity between the studies and the largest study in the review showed no difference between ultrasound guidance and CTT. A further study demonstrated no difference in ongoing pregnancy rates between 2D vs 3D ultrasound guided embryo transfers, however this study did not use LBR as an endpoint and did not report on procedure duration/difficultly, both of which are known to impact ET success rates.
Study design, size, duration: This was a prospective, open labelled randomised controlled trial comparing superiority between two techniques for ET (4DUS vs CTT). A total of 320 (n=160/group) patients were recruited using computer generated randomisation that were centrally distributed in consecutive sealed opaque envelopes between July 2018 to December 2019. Main outcomes were clinical pregnancy rate (CPR) and LBR. Following the procedure, participants completed a survey based on their comfort and satisfaction.
Participants/materials, setting, methods: Inclusion criteria included single blastocyst transfer and a normal uterine cavity. Participants were recruited and randomized on the day of ET. Those allocated to the CTT group, had their embryo transferred without ultrasound, depositing the embryo 6cm from the external os. Those in the 4DUS group had their ET using transvaginal 4D ultrasonography and had their embryos deposited at the maximal implantation point (MIP).
Main results and the role of chance: Results were available from a total of 295 women (8% attrition rate, CTT n=153; 4DUS n=142)).

No demographic differences between the two groups (CTT and 4DUS) were noted including age (p=0.05), BMI (p=0.29), duration of infertility (p=0.94), type of infertility (p=0.68) or embryo quality (p=0.89). All the 4DUS and 95% of the CTT group were performed by the same practitioner.

The 4DUS resulted in significantly higher CPR (50% vs 36% p=0.015, OR 1.78 (1.12-2.84)) and LBR (41%vs 28%, p=0.021, OR 1.77 (1.09-2.87)).

There were no statistically significant differences between miscarriage (p= 0.494), pregnancy of unknown location (p= 0.141) or ectopic pregnancy rates (p=0.958) between the two groups. The 4DUS process took significantly longer time compared with the CTT procedure (15.7 vs 10.2 minutes respectively, p<0.01). The results of the survey showed no statistical difference between patient comfort (p=0.17) or satisfaction (p=0.08) between the groups however there were significantly more positive comments in the 4DUS (p<0.01). In the 4DUS group there was no difference in mean endometrial thickness (P=0.186) or endometrial volume (p=0.836) between pregnant and non-pregnant patients.
Limitations, reasons for caution: Due to the nature of this trial we were unable to blind the participants due to the obvious differences between the methods. Wallace catheters were used for the CTT and Kitazato catheters for the 4DUS, whilst a methodological weakness; previous meta-analysis has not shown any difference between different soft catheters.
Wider implications of the findings: LBRs, when utilizing 4DUS, are significantly higher than the current UK average (41%vs22-23%) and significantly higher than CTT. 4DUS allows for superior imaging of the uterine cavity, tailoring the embryo deposition point specifically to the patient. Further RCTs are required to confirm that 4DUS is the superior technique for ET.
Trial registration number: ISRCTN79955797 ,IRAS 202857

O-182 Higher risk of preeclampsia and pregnancy-induced hypertension with artificial cycle for Frozen-thawed Embryo Transfer compared to ovulatory cycle or fresh transfer following In Vitro Fertilization

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Study question: Is there an increased risk of preeclampsia after Frozen-thawed Embryo Transfer(FET) compared to In Vitro Fertilization-fresh transfer(IVF-fresh-ET) according to endometrial type of preparation for FET?
Summary answer: The frequency of preeclampsia and hypertension were significantly higher in the group of artificial cycle (AC-FET) compared to ovulatory cycle (OC-FET) and fresh-ET (*P*<0.0001).
What is known already: Risks of maternal morbidity are known to be reduced in pregnancies resulting from FET compared to fresh-ET except for the risk of preeclampsia, that was reported to be significantly higher in pregnancies resulting from FET compared to fresh-ET or spontaneous conception. Most recent studies demonstrate an equal live birth rate with either OC-FET or AC-FET preparation. Few studies compared the maternal vascular morbidities with the two hormonal environments that preside over the early stages of embryonic development: OC (major role of the corpus luteum) and AC (prolonged hormone replacement with high doses of estrogen and progesterone).
Study design, size, duration: We conducted a 2013-2018 French nationwide cohort study comparing maternal vascular morbidities in 3 groups of single pregnancies> 22 weeks of gestation (WG): FET with AC or OC preparation, and IVF (conventional or ICSI)-fresh-ET.Data were extracted from the French National Health System database (>99% of national deliveries) in which all hospitalizations are registered, containing information on patient characteristics, diagnoses and treatments. Records were merged anonymously. Access to the database was legally approved.
Participants/materials, setting, methods: 68 025 deliveries were included: fresh-ET(n=48 152), OC-FET(n=9 500), AC-FET(n=10 373). In OC-FET, a luteal phase support with progesterone was administered for maximum 6 WG if pregnancy. In AC-FET, progesterone was co-administered with estrogen until 12 WG. Embryos were transferred at cleavage or blastocyst stage.

Vascular disorders were recorded if hospitalization for preeclampsia/eclampsia or hypertension (history of hypertension excluded). Maternal characteristics were included in multivariate analysis. Adjusted odds ratios(aOR) and 95% confidence intervals(CI) were estimated.
Main results and the role of chance: *Maternal characteristics:* In multivariate analysis, patients in the FET groups were older (33.4 years (std=4.3) *vs.* 33.2 years (std=4.4) for fresh-ET, respectively, *P*<0.0001), less often primiparous (aOR=0.68[0.66-0.71], *P*<0.0001) or smokers (aOR=0.84[0.75-0.95]) or with premature ovarian insufficiency (POI) (aOR=0.68 [0.58-0.79]), more frequently with polycystic ovaries (PCOS) (aOR=1.25[1.12-1.39]) and comparable for obesity or diabetes.

In FET groups, 52.2% were AC-FET. There was no difference for maternal age, parity, obesity, smoking, history of diabetes between AC and OC-FET. Endometriosis (aOR=1.26[1.16-1.38]), PCOS (aOR=1.79[1.50-2.15]) and POI (aOR=2.0[1.48-2.72]) were more frequent in AC-FET.

Risks of vascular disorders: The rate of preeclampsia (5.3% *vs.* 2.3% *vs.* 2.4%, respectively, *P*<0.0001) and hypertension (4.7% *vs.* 3.4% *vs.* 3.3%, respectively, *P*=0.0002) was significantly higher in AC-FET *versus* OC-FET and fresh-ET.

In multivariate analysis, the risk of preeclampsia increased with age, primiparity, obesity, diabetes and POI. The risk was higher in AC-FET *versus* OC-FET (aOR=2.42 [2.06-2.85]) and fresh-ET (aOR=2.43[2.2-2.7]), *P*<0.00001. No difference was found between OC-FET and fresh-ET (*P*=0.91). The risk of pregnancy-induced hypertension increased with age >40, primiparity, smoking, obesity and diabetes and was higher in AC-FET *versus* OC-FET (aOR=1.50[1.29-1.74],

P<0.0001) and fresh-ET (aOR=1.50[1.35-1.67], *P*<0.0001) and not different between OC-FET and fresh-ET (*P*=0.86).
Limitations, reasons for caution: While the strength of this study relies in the number and exhaustiveness of subjects analysed, its limitations are its retrospective and register-based nature that did not enable to refine the risk according to details of techniques and treatments in each group.
Wider implications of the findings: This large nationwide cohort study highlights 2 important information for physicians : i) the possible deleterious role of high supra-physiological and prolonged doses of estrogen-progesterone supplementation on vascular pathologies ii) the protective role of the corpus luteum present in stimulated or spontaneous OC for their prevention.
Trial registration number: Not applicable

O-183 Increased Risk Of Hypertensive Disorders Of Pregnancy In Hormone Replacement Therapy Cycle - A Multicenter Cohort Study In Frozen Blastocyst Transfer In Ovulatory Women

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Study question: Is hormone replacement therapy cycle (HRT) associated with a higher risk of adverse perinatal outcomes than natural cycle (NC) during frozen embryo transfer (FET)?
Summary answer: Higher rates of hypertensive disorders of pregnancy (HDPs) and macrosomia were detected in HRT-FET as compared to NC-FET in ovulatory women.
What is known already: Live-birth rates after HRT-FET and NC-FET are found to be comparable. Recent data showed that pregnancies following HRT-FET are associated with higher risks of HDPs. However, the results might be influenced by selection bias as patients with ovulation disorder were more prone to receive HRT than ovulatory women. As is known, patients with ovulation disorder might have more endocrine disturbances than ovulatory women, which could be associated with adverse obstetrical outcomes.
Study design, size, duration: Four large reproductive medical centers in Guangdong province, Southeast of China, took part in this multicenter retrospective cohort study. Patients with regular cycles (25-35 days), who underwent either HRT or NC blastocyst FET and delivered after 20 weeks of gestation between January 2017 and December 2019 were analyzed. Preimplantation genetic testing (PGT) cycles, multiple pregnancies and cases with type II diabetes or preconceptional hypertension were excluded. Each patient only contributed one cycle per cohort.
Participants/materials, setting, methods: Treatment cycles from each patient were linked to their obstetrical medication record and a comprehensive chart review was done to investigate their perinatal outcomes. Maternal and neonatal outcomes were compared between NC-FET and HRT-FET cycles. Multiple logistic regression analyses were performed to adjust the confounding factors including baseline demographics (maternal age, BMI, education level, parity, type of infertility and cause of infertility), as well as IVF characteristics (insemination method and embryo cryopreservation duration).
Main results and the role of chance: A total of 406 cases from NC-FET and 602 cases from HRT-FET were included. A multiple logistic regression analysis showed that pregnancies after HRT-FET had increased odds of HDPs [adjusted odds ratio (aOR) 2.44, 95% confidence interval (CI), 1.39–4.29] in comparison to pregnancies after NC-FET. Singletons born after HRT-FET were at increased risk of macrosomia compared to NC group (aOR 2.74, 95%CI 1.10–6.87). No significant difference could be seen regarding other obstetrical complications including gestational diabetes, placenta previa, placental abruption and postpartum hemorrhage between HRT-FET and NC-FET. No significant differences were noticed for preterm birth and low birthweight between the different endometrial protocols.
Limitations, reasons for caution: Our study was retrospective in nature, and some cases were excluded due to missing data.
Wider implications of the findings: Pregnancies following HRT-FET are associated with higher risks of HDPs and macrosomia in ovulatory women. Physicians should be cautious on the decision of the endometrium preparation for FET, especially for those who can ovulate normally.
Trial registration number: 2018YFC100310

O-184 Maternally inherited differences in mitochondrial DNA genotype between ART and spontaneously conceived individuals associate with low birthweight

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[7]Maastricht University Medical Center, Department of Toxicogenomics- Clinical Genomics Unit- Maastricht University- Maastricht- The Netherlands- MHeNs School for Mental Health and Neuroscience- Maastricht University- GROW School for Oncology and Develop,

Study question: Can mitochondrial DNA (mtDNA) variants explain the differences in birthweight between ART and spontaneously conceived (SC) individuals and how do they originate?
Summary answer: Children born after ART carry more frequently a different mtDNA variant composition, both maternally inherited and *de novo*, which are predictive of their birthweight percentile.
What is known already: Children born after ART show an increased risk of lower birthweight and of developing a mild abnormal cardio-metabolic profile later in life. Variation in the mtDNA associates with overall health in the general population, including cardio-metabolic fitness, and can result in changes in mitochondrial function. We hypothesized that mitochondrial DNA variants could explain the differences in birthweight between ART and SC individuals and that these differences may result from maternal transmission and/or from the ovarian stimulation (OS) used in ART.
Study design, size, duration: We deep-sequenced the mtDNA of 472 individuals of who 283 ART and 189 SC, 182 mother-child pairs and 113 single oocytes from both natural menstrual cycles and OS cycles. The mtDNA was compared between groups and Fisher linear discriminant analysis was used as predictive model for the birthweight percentile.
Participants/materials, setting, methods: Mitochondrial DNA was enriched by long-range PCR and subsequently sequenced on an Illumina platform. mtDNA server and MuTect were used for variant calling for variants with a load higher than 1.5%, versus the reference NC_012920.1. An orthogonally rotated factor analysis was used to reduce the dimensionality of the studied dependent variables in the complex data of the heteroplasmic variants.
Main results and the role of chance: ART individuals carried more frequently haplogroup U4 (p=0.004) and component analysis indicated that they carry a different mtDNA heteroplasmic variant composition than SC individuals (p=0.01), driven by non-synonymous protein-coding and rRNA-coding variants. These differences were also predictive of the risk of a lower birthweight percentile, especially for the SC children, together with the absence of haplogroup T, the presence of homoplasmic tRNA-variants, pregnancy-induced hypertension and the embryo culture medium used. The differences in heteroplasmic variation observed in the ART children resulted from both maternal transmission (p=0.03) and *de novo* mutagenesis (p=0.02). Mothers of ART children showed a similar mtDNA genotype as their children and differed in the same variant composition when compared to the mothers of SC children (p=0.03). Furthermore, the comparison of oocytes from the same donors retrieved in natural menstrual cycles and after one OS cycle showed that OS does not increase *de novo* mutagenesis. Additionally, clinical parameters such

as the total dosage of FSH units, the number of oocytes retrieved, and maternal age did not show any correlation with the differences observed in ART individuals.
Limitations, reasons for caution: This study is observational with no functional tests being performed.
Wider implications of the findings: We demonstrate an association between a lower birthweight percentile and a mtDNA variant composition which is more frequently carried by ART children. These non-disease associated mtDNA variants could cause a suboptimal mitochondrial function affecting the birthweight. Long-term health consequences of these differences remain to be further elucidated.
Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 64: HOW EFFECTIVE IS HIGH QUALITY INFORMATION IN SUPPORTING FERTILITY DECISION MAKING?

30 June 2021 Stream 3 17:00 - 18:00

O-185 Evaluation of the decision-making process of girls with Turner syndrome and their parents considering ovarian tissue cryopreservation

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Study question: What are the experiences with the decision-making process of girls with Turner syndrome (TS) considering ovarian tissue cryopreservation (OTC), their parents and healthcare providers?
Summary answer: Offering a new option to preserve fertility in TS caused unrealistic hope leading to challenges for healthcare providers to fulfil the ideal of informed consent.
What is known already: Due to premature ovarian insufficiency, girls with TS have only a small chance of genetic offspring. OTC might increase these odds. Healthcare providers and scientist are still cautious in offering OTC to girls with TS because of the many uncertainties regarding OTC in this patient group. Hence, OTC is now offered to girls with TS between 2 and 18 years old in a research setting: the TurnerFertility study.
Study design, size, duration: A retrospective qualitative study consisting of a survey and focus groups. Within a year after counselling, families (n=132) received a survey with 30 questions regarding their experiences with the decision-making process and also an invitation for a focus group. The focus groups were conducted between January and October 2019 and lasted 51-84 minutes. The topic lists were based on literature research and survey results. Results were analysed following a thematic analysis approach.
Participants/materials, setting, methods: This is a sub-study of the prospective intervention study within an academical medical centre. Focus groups were composed through purposive sampling. Focus group 1 (FG1) consisted of five gynaecologists involved in counselling, FG2 with seven paediatricians who referred girls for counselling, FG3 with nine parents of girls with TS between 2 and 12 years old and FG4 with three parents of girls with TS between 13 and 17 years old.
Main results and the role of chance: 90% of survey respondents appreciated counselling regarding fertility options and considered it an enrichment of existing healthcare. The individual consultation was rated as most contributing by 66% of the survey respondents, followed by the information meeting (37%) and decision aid (3%). The focus groups revealed that many had not discussed options for future parenthood with a healthcare provider before. Girls with TS and their parents indicated that the option of OTC raised hope for future genetic offspring, and at once made them feel like they had no choice but to take this chance. The small chance of success did not influence the decision for families who opted for OTC. Some parents who had to decide for their young daughter accepted OTC to give their daughter the option to decide herself whether to make use of the cryopreserved tissue later in life. Gynaecologists found it challenging to truly make families grasp a realistic perspective of OTC in TS and the associated mental and physical risks. Gynaecologists and paediatricians struggled with conflicting moral principles of non-maleficence against respect for autonomy: healthcare providers recognized the scientific relevance for the TS population, while it felt inconsistent with the disproportionate burden for some individual patients.
Limitations, reasons for caution: Because there was no validated survey for this topic in TS, we developed a survey based on literature research and experiences of a dedicated TS team. Among the survey responders and focus group participants a greater proportion decided for OTC compared to the overall counselled group (75% vs 60%).
Wider implications of the findings: This study gives insight in the issues to consider when implementing new technologies regarding fertility, in which parents have to decide for their child, where it is expected that anticipated decision regret plays a major role, or where healthcare providers experience conflicting duties as scientist and physician.
Trial registration number: not applicable

O-186 From knowing to helping-seeking: An examination of FertiSTAT and infertility-related treatment behaviours among Chinese female in Hong Kong

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Study question: Do fertility health risks predict Hong Kong Chinese women's perceived susceptibility to infertility and engagement in treatment behaviours?
Summary answer: Presence of age-related, medical and lifestyle risk factors did not translate into actual infertility-related treatment behaviours among reproductive-aged women.
What is known already: The Health Belief Model posits that perceived susceptibility to a health condition determines whether one would engage in risk-reducing behaviour. There is yet to be large-scale studies that investigate the rate of fertility health risks among Hong Kong Chinese women. FertiSTAT is an empirically-derived tool that assesses risks to female fertility and provides personalised guidance on risk reduction. Using a Chinese version of the FertiSTAT, this study investigates the fertility risk profile of reproductive-aged women, for whom timely treatment-seeking would be especially pertinent. Additionally, we explore whether fertility health risks translate into awareness of personal susceptibility and actual treatment behaviours.
Study design, size, duration: This is a cross-sectional online survey conducted in Hong Kong between July and August 2020.
Participants/materials, setting, methods: Five-hundred twenty-nine childless women (mean age = 29±6.6) were recruited to the study through community network and social media. Respondents completed the fertility status awareness tool (FertiSTAT) and questions relating to reproductive intention and help-seeking behaviour. Logistic regression analyses were conducted to explore the impact of lifestyle-, medical- and age-related fertility health risks on perceived susceptibility and infertility-related treatment behaviours.
Main results and the role of chance: The fertility health risks most frequently reported by this sample of women were menstrual cycle irregularity (35%) and having stress one cannot cope with (26%). Among women who were trying to conceive (n=67), 40% had been trying for over 12 months, exceeding the critical threshold for risks of infertility. More than one third of women suspected that they had an underlying risk of infertility, however only a small minority had ever taken steps to investigate the issue further by seeking medical support. Binary logistic regression revealed that women who experienced severe menstrual pains (B=-2.70, P<.05) or who had excessive caffeine consumption

(B=-3.27, P<.05) were less likely to suspect that they had an underlying risk of infertility compared to when they had other risk factors. Prolonged time trying to conceive (B=1.52, P<.05) significantly predicted seeking gynaecological exam; whereas none of the fertility health risks had an impact on seeking medical consultation from a TCM practitioner.

Limitations, reasons for caution: Recruitment through social media may bias the sample towards women with greater access to online fertility-related information, posing questions on how generalizable the current findings are to less resourceful individuals. The relatively young age of the sample may also underestimate the rate of fertility health risks in the population.

Wider implications of the findings: Targeted public education initiatives are needed to raise awareness of the impact of not only age-related, but also medical and lifestyle risk factors on reproductive potential. Reproductive-aged women with fertility health risks should be alarmed of suspected infertility and be encouraged to seek proper medical examinations and treatments.

Trial registration number: not applicable

O-187 Smartphone application improves fertility treatment-related literacy: A large-scale surveillance and randomized controlled trial in Japan

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Study question: Can providing quality-assured fertility-related information via a smartphone application improve fertility- and treatment-related literacy among smartphone application users?

Summary answer: Provision of quality-assured fertility-related information via a smartphone application contributed to enhancing fertility- and treatment-related literacy among the smartphone application users.

What is known already: For infertility patients, the interpretation of examination results may be overly complicated and complex, and patients may have difficulty in making sense of their own fertility problems. Accessing and learning about fertility-related information using the Internet via smartphone is reasonable; however, the information does not always reflect evidence-based recommendations and low-quality information may lead to adverse effects on users; thus, innovative methods to provide both accessible and high-quality information are desired.

Study design, size, duration: We performed a randomized control-group pretest posttest study and 4,137 smartphone application users were invited to participate between June 18 and 25, 2020. Participants' fertility treatment-related literacy were assessed with a pretest that comprised of 28 questions and participants were allocated with stratified randomization to either intervention or control group. The intervention comprised a one-week smartphone application-based provision of information on fertility- and treatment-related information and the control group received general information about women's healthcare.

Participants/materials, setting, methods: The 3,765 participants (91.0 %) who responded were randomly allocated into either the intervention group (N=1883) or the control group (N=1882). Characteristics of participants appeared similar between the groups reflecting that the randomization was successful in producing a balance in baseline characteristics. Effectiveness of intervention was assessed using pretest-posttest analysis. Ethical approval was obtained from the Institutional Review Board of the National Center for Child Health and Development of Japan (approval number: 2019-184).

Main results and the role of chance: The posttest was completed by 659 participants (17.5%), and finally 207 participants in the intervention group and 222 participants in the control group were available for pretest-posttest analysis. Demographic characteristics of these participants appeared similar between the groups. In comparing the demographic characteristics of participants who did and did not complete the posttest, there were significant differences between the two groups in age, overall test score, proportion living with a partner, and action for pregnancy. For the posttest, the overall mean test scores were significantly higher in the intervention group compared to the control group (P = 0.0082). Interestingly, we also observed that posttest scores were significantly improved compared to pretest scores in both the intervention group and control group (P<0.001). When examining by specific test question, the proportion answering correctly appeared to generally increase at posttest compared to pretest for intervention (P<0.001) and control (P<0.001) groups. There was over 10% improvement in 7 questions, and particularly, over 20% improvement for a question about clinical significance of anti-Müllerian hormone. Furthermore, directly comparing the difference in posttest versus pretest scores between the two groups showed, on average, greater improvements in the intervention group than the control group (P<0.001).

Limitations, reasons for caution: As the intervention was educational material, it was not possible to blind participants to intervention group assignment. We were not able to monitor the participants when completing the tests; thus, whether they accessed other resources could not be addressed.

Wider implications of the findings: Providing information through a smartphone application can be considered acceptable since retrieving information through a smartphone application is in line with the current modern day lifestyle. A smartphone application may offer alternatives such as chatbots and movie-based learning, and they have the potential to increase the effectiveness.

Trial registration number: UMIN Clinical Trials Registry number UMIN000040721.

O-188 Participation in a video-based fertility awareness program advances the desire to have children sooner

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Study question: Participating in a fertility awareness program accelerates the intention to have children 6 to 12 months after video- fertility awareness?

Summary answer: In combination with positive childbearing motivation, those in the video condition anticipated their intentions to have children at 6-12 months follow-up

What is known already: Fertility awareness education and initiatives have been focused on increasing fertility awareness and knowledge. However, the role of fertility awareness on reproductive decisions is less explored in literature. There are few studies showing that participating in fertility awareness education is related to higher intentions to have children or decreased time until trying to conceive regarding beforehand planned. Since intentions seem to be a good predictor of behaviour, we were interested in exploring the variables which might predict higher intentions to have children after participating in a fertility awareness education study based on a video intervention

Study design, size, duration: This study is part of a randomised controlled trial initiated in October 2016. Participants were randomly allocated into the intervention (IG) and control group (CG) at baseline (TO). The follow-up was evaluated 6 to 12 months later. IG participants were exposed to a 5-min video delivering information on age-related fertility decline, infertility risk factors, and pregnancy chances after having filled-in the T0 questionnaire: Participants in CG received no stimulus.

Participants/materials, setting, methods: Three hundred sixty-five individuals (65% women) were recruited through social media, gynaecology clinics, and religious pre-marital courses. From those, 128 responded to follow-up

(between 6 and 12 months). Childbearing Motivation Scale (positive and negative) and a question about childbearing timing intentions were used. Regression analysis was conducted to explore the role of video intervention and childbearing motivation on childbearing timing intention.

Main results and the role of chance: Participants were, on average, 29.5 years old (SD=5.13). From baseline to 6-12 months later, participants significantly anticipated their desire to have children ($p<.005$). Correlations showed that childbearing timing intention was positively and significantly associated with the following subscales of the positive motivations: personal fulfilment, continuity, and couple relationship; negative motivations were not associated with the childbearing timing intention. The regression analysis revealed that only the childbearing motivation regarding couple relationship (having a child would strengthening partnership ties, fulfilling partner's project, growing as a couple, fulfilling a shared project) and the visualization of the educational video significantly contributed to predicting at follow-up the increased desire to have children sooner at follow-up.

Limitations, reasons for caution: Other factors, not considered, might contribute to the prediction of childbearing motivation timing. Although we had randomly allocated people to CG and IG, the high attrition rate may limit the generalization of our results.

Wider implications of the findings: This study seems to indicate that watching an educational video about fertility awareness might contribute to a significant change in childbearing timing. In addition, the importance of the couple relationship is highlighted. These findings reinforce the importance to assist people with informed reproductive decisions in social and healthcare settings.

Trial registration number: NCT02813993

SELECTED ORAL COMMUNICATIONS

SESSION 65: MALE AND FEMALE FERTILITY PRESERVATION: NEW INSIGHTS FROM THE LABORATORY

30 June 2021 Stream 4 17:00 - 18:00

O-189 Male fertility restoration by direct transplantation of human infant testicular cells into infertile recipient mouse testis

D. Wang[1], S. Hildorf[2], L. Dong[1], S.E. Pors[1], L.S. Mamsen[1], E.R. Hoffmann[3], D. Cortes[4], J. Thorup[2], C.Y. Andersen[1]

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[4]Copenhagen University Hospital Hvidovre, Department of Pediatrics, Copenhagen, Denmark

Study question: Is colonization of human gonocytes and spermatogonial stem cells (SSCs) directly transplanted to seminiferous tubules of busulfan sterilised mice testis during an 8-week period feasible?

Summary answer: Gonocytes and SSCs from infant boys can settle on the basal membrane and form germline stem cell colonies in the seminiferous tubules of recipient mice.

What is known already: The neonatal or immature animal provides higher populations of gonocytes and/or SSCs than adults, and the number of transplanted donor SSCs directly affects the colonization rate of the recipient testes. Along with SSC transplantation restoring the recipient's spermatogenesis, donor gonocyte was also reported to be capable of establishing spermatogenesis in rodents.

Study design, size, duration: Transplantation of human testicular cells including gonocytes and SSCs into seminiferous tubules of infertile recipient mice. We included 10 infant testis biopsies from which single-cell suspension was transplanted individually into the seminiferous tubules of 10 immunodeficient mice. The immunodeficient mouse testes were injected with busulfan to deplete germ cells. Four weeks later, we did the xenotransplantation. Then after eight weeks, we collected all mouse testes to do further analysis.

Participants/materials, setting, methods: Testis biopsies were obtained from cryptorchid boys undergoing orchidopexy. After enzymatic digestion of the testis biopsies, dissociated single-cell suspensions were pre-labeled with a green fluorescent dye. Then the single-cell suspensions were transplanted into seminiferous tubules of the infertile recipient mice. Eight weeks later, the presence of gonocytes and SSCs was determined by immunohistochemistry and whole-mount immunofluorescence.

Main results and the role of chance: Without *in vitro* propagation, naturally enriched human germline stem cells settled on the basal membrane of seminiferous tubules and survived in the mouse testes at least for two months demonstrating that human gonocytes and SSCs were capable of colonizing the recipient mouse seminiferous tubules.

Limitations, reasons for caution: The study samples were from infant boys with undescended testes that were more likely to contain gonocytes. It was not possible to determine which germ-cell type at transplantation resulted in the detected gonocytes and SSC colonies after xenotransplantation. Transplantation of gonocytes may include the potential risk of stem cell-related malignancy.

Wider implications of the findings: Without *in vitro* propagation, male germline stem cell-based transplantation could provide a relatively safe therapeutic treatment for prepubertal boys with cryptorchidism and boys diagnosed with cancer. This method could also facilitate clinical translation.

Trial registration number: not applicable

O-190 Comparison between effects of exposure to platinum-based chemotherapeutics (cisplatin and carboplatin) on Sertoli cell number and functions in immature human testicular tissues

G. Matilionyte[1], M.D. Tharmalingam[1], I. Sanou[1], F. Lopes[1], R.A. Anderson[1], R.T. Mitchell[1]

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Study question: Does exposure to either cisplatin or carboplatin have a damaging effect on the Sertoli cell population in the immature human testicular tissues?

Summary answer: Exposure to cisplatin or carboplatin did not appear to have a major effect on Sertoli cell number or function in the immature human testicular tissues

What is known already: Long-term survival rates for children with cancer are more than 80%. However, childhood cancer treatment may result in subsequent infertility. Cisplatin is one of the most commonly used drugs for childhood cancers. Carboplatin, a second generation platinum drug, is administered at 10-times the dose of cisplatin and is believed to be less gonadotoxic. In our recent publication we have shown that exposure to both cisplatin and carboplatin acutely reduce the germ cell number in immature human testicular tissues. However, it is not known how cisplatin and carboplatin affect Sertoli cell number and function.

Study design, size, duration: In-vitro culture of human fetal and pre-pubertal testicular tissues was utilised. Tissue pieces were cultured for 1-3 days prior to exposure to clinically-relevant doses of chemotherapeutics or vehicle control for 24hrs in two sets of experiments: 1) 0.5 or 1 µg/ml cisplatin and culture ended at 24 and 96hrs post-exposure (fetal only); 2) 0.5 µg/ml cisplatin or 5 µg/ml carboplatin until 72 (both fetal and pre-pubertal) and 240hrs post-exposure (fetal only).

Participants/materials, setting, methods: Testicular tissue fragments from second trimester human fetal (14-22 gestational weeks; n=3-6) or pre-pubertal patients (1-8years old; n=5) were cultured in a 'hanging drop' system. Quantification of Sertoli cell number (cells per cord/tubular area (mm^2)) was performed on sections stained for expression of SOX9. Culture medium was collected to measure levels (ng/ml) of Anti-Mullerian hormone (AMH) and Inhibin B using ELISA. Statistical analysis was performed using two-way ANOVA to account for inter-individual variation between fetuses/patients.

Main results and the role of chance: Quantification of positively stained Sertoli cells showed that exposure to both doses of cisplatin had no effect on Sertoli cell number at 24 and 96hrs post-exposure. No changes in AMH and inhibin B levels were observed at these time-points. Comparison between cisplatin- or carboplatin-exposed human fetal testicular tissues showed no

difference in Sertoli cell numbers at either 72hrs or 240hrs post-exposure. No difference in Sertoli cell number was observed in pre-pubertal testicular tissues exposed to either cisplatin or carboplatin at 72hrs post-exposure.
Limitations, reasons for caution: Human fetal and pre-pubertal testis tissue is of limited availability, thus, sample sizes used in this study were relatively low. 'Hanging drop' culture might not recapitulate all in-vivo aspects of immature testis microenvironment.
Wider implications of the findings: Exposure to cisplatin or carboplatin did not affect Sertoli cell number in the immature human testicular tissues. Taken together with our recent publication, this suggests that these two platinum-based chemotherapeutic agents cause direct damage to germ cells. Functionality of Sertoli cells in chemotherapy-exposed tissues need to be further investigated.
Trial registration number: not applicable

O-191 Assessing the use of tumour-specific DARPin-toxin fusion proteins for ex vivo purging of cancer metastases from human ovarian cortex tissue fragments before autotransplantation

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Study question: Is it possible to eradicate cancer cells from ovarian cortex by using tumour-specific designed ankyrin repeat protein (DARPin)-toxin fusion proteins, without compromising the ovarian tissue?
Summary answer: Purging ovarian cortex *ex vivo* from experimentally induced breast cancer tumour foci is possible by tumour-targeted DARPin-toxin fusion proteins trough inhibition of protein synthesis.
What is known already: Ovarian tissue cryopreservation and autotransplantation is a successful technique for fertility restoration in cancer patients. The procedure is not without risk since malignant cells may still be present in the graft. Procedures to detect cancer cells render the tissue fragment useless for autotransplantation. Strategies to circumvent this problem such as *in vitro* maturation of follicles or the construction of artificial ovaries are pursued but are still experimental. Alternatively, we have shown *ex vivo* purging of ovarian cortex is possible by elimination of rhabdomyosarcoma after treatment with verteporfin. This allows treatment of cortex fragments before autotransplantation without compromising ovarian tissue integrity.
Study design, size, duration: Human ovarian cortex fragments harbouring breast cancer tumour foci were exposed for 24 h to DARPins fused to the translocation and catalytic domain of *Pseudomonas aeruginosa* exotoxin A (DARPin-toxin fusion proteins) targeting EpCAM or HER2. After treatment with the DARPin-toxin fusion proteins the tissue was cultured for an additional 6 days to allow any remaining tumour cells to form foci. In addition, the functional integrity of the ovarian tissue was analysed after purging.
Participants/materials, setting, methods: Breast cancer cell lines expressing different levels of EpCAM and HER2 were introduced in human ovarian tissue to form tumour foci. After purging with DARPin-toxin fusion proteins, the presence of any remaining cancer cells in the tissue was analysed with (immuno)histochemistry and RT-qPCR. Possible detrimental effects on the viability of ovarian cortex and follicles were determined by (immuno)histology, a follicular viability assay and an assay to determine the *in vitro* growth capacity of small follicles.
Main results and the role of chance: Ovarian cortex harbouring EpCAM-positive breast cancer cells showed a significant decrease in the number of tumour foci after treatment with the EpCAM-targeted DARPin-toxin fusion proteins. Although exposure to the EpCAM-specific DARPin had no effect on morphology or viability of follicles, a decrease in oocyte viability after *in vitro* growth experiments was observed, presumably due to low level expression of EpCAM on oocytes. In contrast to the EpCAM-specific DARPin-toxin fusion protein, the DARPin-toxin fusion protein targeting HER2 had no detrimental effects on morphology, viability or *in vitro* growth of follicles while foci of HER2-positive breast cancer cells were severely affected as indicated by the presence of apoptotic bodies, tumour cell remnants and the absence of viable tumour cells. The histological results after purging with the HER2-specific DARPin-toxin fusions proteins were confirmed by RT-qPCR, showing a decrease to basal levels of HER2 mRNA in the ovarian cortex tissue.
Limitations, reasons for caution: The effect of DARPin-toxin fusion proteins depends heavily on the expression of their target on the cancer cell. The target protein should not be expressed by ovarian cortex as this may lead to tissue damage. The functional integrity of ovarian cortex after the treatment requires further investigation *in vivo*.
Wider implications of the findings: Purging metastases from ovarian cortex without harming ovarian tissue is possible by targeting tumour specific surface expressed antigens with DARPin-toxin fusion proteins. Purging ovarian cortex tissue with DARPin-toxin fusion proteins provides a feasible therapeutic strategy to prevent reintroduction of cancer by autotransplantation in case of malignancies expressing tumour-specific surface markers.
Trial registration number: not applicable

O-192 Modulating hypoxia and oxidative stress in human ovarian tissue xenografts using adipose tissue-derived stem cells

L. Cacciottola[1], T.Y.T. Nguyen[1], C.A. Amorim[1], J. Donnez[2], M.M. Dolmans[3]

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Study question: To investigate whether adipose tissue-derived stem cells (ASCs) modulate hypoxia and oxidative stress in human ovarian tissue transplants to reduce early follicle loss.
Summary answer: ASCs protect the follicle pool by mitigating the hypoxia-related response through HIF1α signaling in human xenografts and enhancing revascularization by ensuring faster tissue reperfusion.
What is known already: ASCs are known for their angiogenic potential and capacity to boost angiogenesis by secreting growth factors and differentiating into vessels in numerous models of wound healing in regenerative medicine. In a 2-step ovarian tissue xenotransplantation involving grafting inside a fibrin scaffold two weeks prior to transplantation, ASCs reduced follicle loss after short- and long-term grafting, as well as abnormal follicle activation, by increasing reoxygenation and revascularization in human xenografts.
Study design, size, duration: Prospective experimental study. Cryopreserved ovarian cortex from five adult women was transplanted to 30 nude mice, with or without ASCs (ASC group; OT group). Ovarian grafts were retrieved on days 3 (n=5), 10 (n=5) and 21 (n=5). One piece of ovarian tissue per patient was fixed for analysis after thawing to serve as non-grafted controls.
Participants/materials, setting, methods: The 10 animals grafted for 21 days underwent in vivo microdialysis evaluation to investigate direct reactive oxygen species (ROS) kinetics. Analyses of ovarian grafts at all time points and non-grafted controls included immunolabeling for double CD34 (revascularization by host and graft components), immunofluorescence for HIF1 (hypoxia-related response), Nrf2 (oxidative stress-related response) and 8OHdG (oxidative stress-related DNA damage), and gene expression (RT-qPCR) for VEGF-A (neoangiogenesis), SOD2 (antioxidant activity) and Nrf1 (mitochondrial biogenesis).
Main results and the role of chance: ROS peaked sooner in the ASC group (day 2, $p<0.0001$) than the OT group (day 10, $p=0.01$) after grafting, indicating earlier tissue reperfusion. Total vascularization was stable in the ASC group at all time points, but lower in the OT group 3 days after grafting ($p=0.01$) due to a drop in both host and graft vascular components. HIF1 expression, detected mainly in follicles, was significantly lower in primordial follicles in the ASC group than the OT group on days 3 ($p=0.008$) and 10 ($p=0.01$). VEGF gene expression rose significantly (around 40x) in both groups on day 3 and persisted significantly longer in the ASC group (10 days) than the OT group (3 days) ($p=0.04$), emphasizing the role of ASCs as enhancers of proangiogenic factors. There was no upturn in the oxidative stress-related response (Nrf2 pathway) nor DNA damage (8OHdG) to follicles in any of the grafted groups over time, while a modest increase in both markers was observed only in the stroma after 21 days. Neither was there any major increase in SOD2 and Nrf1 gene

expression, suggesting no significant activation of the Nrf2 pathway for cytoprotection from oxidative stress.

Limitations, reasons for caution: Although Nrf2 signaling activation was detected in human granulosa cell cultures in increasing ROS concentrations, our findings did not confirm its role in tissue damage modulation after ovarian tissue transplantation. Further studies may evidence the involvement of other pathways that modulate oxidative stress after transplantation.

Wider implications of the findings: The role of ASCs in protecting the follicle pool appears to be related to a decrease in hypoxia and faster ovarian graft revascularization and reperfusion, sustained by an increase in VEGF for a longer period after grafting. There was no evidence of oxidative stress-related damage, irrespective of the transplantation strategy.

Trial registration number: ‘

human reproduction

ESHRE 2021 / Oral presentations

INVITED SESSION

SESSION 66: THE ENDOMETRIUM IN THE 21ST CENTURY

01 July 2021 Stream 1 08:30 - 09:30

O-065 The naughty cells of the endometriumxx

C. Gargett[1]

[1]*Hudson Institute of Medical Research, The Ritchie Centre, Clayton, Australia*

Abstract text

Stem/progenitor cells are the naughty cells of the endometrium! The term "naughty" has a number of connotations, one being immaturity which I will apply to the rare stem/progenitor cell populations hiding in the endometrium, where they have eluded scientists for so long. Despite their rarity, these immature cells have the capability of growing up and differentiating into the functional cells of the endometrium, producing their progenies in the process. The self-willed human endometrial epithelial progenitor cells (eEPC) and mesenchymal stem cells (eMSC) first revealed themselves through their clonogenic activity, shunning their mates and setting up clones of cells on their own. Their risqué production of identical copies of themselves ensures their continuity, much to the chagrin of their mature counterparts. They are sneaky and can produce large numbers of mature progeny, but rarely proliferate themselves preferring to take life easy and do little. They also spit out viability dyes (Hoechst) at a greater rate than mature endometrial cells to become Side Population (SP) cells.

A number of approaches have been used to tame these naughty endometrial stem/progenitors. In order to determine the identity and location of these elusive cells, specific markers had to be found. The immature endometrial epithelial progenitor cells play tricks with the specific markers they express. For example, clonogenic eEPC are N-cadherin+, an epithelial mesenchymal transition marker, found by unbiassed gene profiling, revealed their hiding place in the bases of glands deep in the endometrial basalis. Similarly, SSEA-1+ basalis epithelial progenitors pirated their marker from mature neutrophils and differentiating human pluripotent stem cells. In mice the stem/progenitor cells like to play chase, with lineage tracing of individual genetically marked cells revealing their location in the intersection zone of the glands and luminal epithelium, and also in the gland bases (*Axin2*+ and *Lgr5*+).

The identity of eMSCs has also been determined by discovery of specific markers, but even here the eMSC play games in human endometrium where sometimes they are pericytes (CD140b and CD146 double positive cells), sometimes perivascular cells (SUSD2+) and sometimes CD34+ cells in the adventitia of blood vessels. They are also adventitial perivascular cells in ovine endometrium, but this time they are CD271+. Mature endometrial stromal cell progeny are also naughty, often pretending to be eMSC, particularly when shed into menstrual fluid, confusing many of their status. Adding further to their misbehaviour, they express the same official MSC surface markers. To get even immature endometrial MSC strike back, claiming immunomodulatory properties in attempt to upstage their mature stromal progeny, also endowed with these properties.

Finally, other endometrial cells such as macrophages may also be naughty as their mischievousness in evading detection can trick us to consider them as stem cells from the bone marrow, masquerading as endometrial epithelial or stromal cells.

Naughty implies behaving badly and I will show data suggesting that stem/progenitor cells may escape the endometrium to cause a nasty disease, endometriosis. They may also become wayward and unruly, invading the myometrium to form adenomyosis. Some naughty epithelial progenitors defiantly pick up mutations to become cancer stem cells and initiate endometrial cancer. They may also malfunction because they do not obey estrogen signalling instructions, failing to proliferate and causing thin unresponsive endometrium. In their naughtiness, they may run away or get totally lost, thereby resulting in Asherman's syndrome. Therefore, for numerous reasons, stem/progenitor cells are the naughty cells of the endometrium.

O-066 The naughty genes and 3D structure of the endometrium.

T. Enomoto[1], **M. Yamaguchi**[1], **K. Suda**[1], **K. Yoshihara**[1]

[1]*Niigata University Graduate School of Medical and Dental Sciences, Department of Obstetrics and Gynecology, Niigata, Japan*

Abstract text

The naughty genes and 3D structure of the endometrium

Takayuki Enomoto, Manako Ymaguchi, Kazuaki Suda, Kosuke Yoshihara

Department of Obstetrics and Gynecology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.

The human endometrium is a highly regenerative tissue and involved in menstruation and implantation of the fertilized egg, giving it a central role in women's reproductive health. However, the regenerative nature of the endometrial glands can lead to the development and progression of "endometrium-related diseases" such as adenomyosis, endometriosis, endometriosis-associated ovarian cancer, endometrial hyperplasia, and endometrial cancer. To clarify the pathogenesis of endometrium-related diseases and develop effective preventative measures and therapeutic strategies, comprehensive understanding of molecular biological linkage between endometrium and endometrium-related diseases was crucially important.

To this end, we focused on genomic alterations of endometrial epithelium which is considered the origin of endometriosis, and sequenced 107 ovarian endometriotic and 82 normal uterine endometrial epithelium samples isolated by laser-microdissection. Intriguingly, several genes recurrently mutated in endometriosis-associated ovarian cancers were frequently mutated in both endometriotic epithelium and normal uterine endometrial glands. In particular, *PIK3CA* mutation was detected in 41% of endometrial epithelium subjects but none of them had shared *PIK3CA* mutations across multiple regions collected from the same individuals. Mutation allele frequencies of somatic mutations in uterine endometrial epithelium samples were also significantly lower than those in ovarian endometriotic epithelium samples, suggesting the heterogeneous genomic compositions in uterine endometrium. To interpret this genomic heterogeneity in uterine endometrium, we focused on endometrial gland, the minimum functional unit of uterine endometrium, and conducted 109 single endometrial glands sequencing. As a result, we unveiled that each gland carried distinct somatic mutations in cancer-associated genes, such as *PIK3CA, KRAS*, and *PTEN,* with high mutant allele frequencies, suggesting the monoclonality of each gland. The presence of cancer-associated gene mutations in histologically normal endometrial glands provides important clues regarding the pathogenesis of endometrium-related diseases.

However, our previous study could not determine the spread of endometrial gland harboring cancer-associated gene mutation because there is a limitation

to two-dimensional assessment of the whole shapes of endometrial gland due to its complicatedly winding morphology. Therefore, we tackled with three-dimensional (3D) assessment of human endometrium. To construct a large picture of endometrial gland structure, we performed tissue-clearing-based 3D imaging of full-thickness human uterine endometrial tissue with the use of light-sheet fluorescence microscopy. Our 3D immunohistochemistry discovered some new and unique 3D morphologies of endometrial glands, including plexus network of glands or occluded glands. Notably, computational analysis of 3D layer clarified that the plexus structure of the glands was mainly located in the stratum basalis and expanded along muscular layer horizontally, similar to the so-called "rhizome of grass". Although previous studies have shown the 3D structure of murine endometrial glands, the bottom of these glands forms a crypt but not a rhizome. This can potentially be explained by the existence of menstruation, which is the crucial difference between the human and murine endometrium. The rhizome structure of endometrial gland in the human endometrium will have a functional advantage over the crypt in terms of the conservation of progenitor/stem cells and regeneration. In addition, some endometrial glands shared the plexus and rose toward the luminal epithelium, suggesting that these glands were the same origin. The rhizome of the endometrium may be a crucial element for understanding the expansion of endometrial glands harboring cancer-associated gene mutations.

Integrated analysis of the naughty gene alterations and the 3D structure in human endometrium will lead to a better understanding of the human endometrium in various fields, including histology, pathology, pathophysiology, reproduction, and oncology.

INVITED SESSION

SESSION 67: MANAGEMENT OF HUMAN RESOURCES

01 July 2021 Stream 2 08:30 - 09:30

O-067 Training of embryologists: How to do it and how to best evaluate it?

B. Woodward[1]

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O-068 Training of clinicians: How to do it and how to best evaluate it

A. Feki, MD- PhD[1], **M. Tatjana**[2], **F. Roy**[3]

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[3]*Liverpool Women's NHS Foundation Trust, Obstetrics and Gynaecology, Liverpool, United Kingdom*

Abstract text

Training of clinicians: How to do it and how to best evaluate it?

Anis Feki, Tatjana Motrenko, Roy Farquharson

Many countries within Europe and EU do not have a specific national agency that provides the necessary structure or governance for appropriate training in Reproductive Medicine (RM). Therefore, The European Society of Human Reproduction and Embryology (ESHRE) has been the responsible agency for accreditation of RM training alongside its sister organization, the European Board and College of Obstetrics and Gynaecology (EBCOG). Both of these organizations are ultimately responsible to and approved by the Union of European Medical Specialties (UEMS), which represents the European Union (EU) governing body for medical practitioners' education.

The Reproductive Medicine subspecialist is a specialist in basic obstetrics and gynecology who has undergone theoretical and practical training in the medical and surgical management of infertility, including assisted reproductive techniques (ART). In general, the comprehensive management of these problems includes both diagnostic and therapeutic procedures allied to continuous audit of outcome.

The global aim is to improve the care of patients with disorders of reproductive function. To reach this target, both ESHRE and EBCOG put in place a program that starts with accreditation of training centers in obstetrics and gynecology and more specifically, specialist training centers in reproductive medicine. Fellows must have a structured program of a minimum of 2 years after completing their basic obstetrics and gynecology syllabus and/or obtaining EFOG European Fellow of Obstetrics and Gynecology) diploma for non-European basic training. When a training fellow in RM has completed their logbook, the fellow will finish by assessing an individual's knowledge and skills by passing an exit-training exam called the EFRM (ESHRE-EBCOG Fellow in Reproductive Medicine) diploma.

The success of and the need for such certification has led ESHRE to structure the training also for reproductive surgeons, embryologists, as well as nurses and midwives. In addition, several countries within the EU already have a national compulsory specialist accreditation system for RM training.

Recent proposals by the EU Commission to build European reference networks across all specialties, including RM may require the recognition of ESHRE/EBCOG–accreditation as an appropriate entry point for consideration of diagnostic and other interventions. Encouraging subspecialists and centers to apply for training accreditation may well prove to be an increasingly attractive option as educational needs across Europe become integrated and formalized through UEMS. ESHRE holds the view that accredited centers/subspecialists for training are beacons of excellence that often go hand in hand with high-quality research.

SELECTED ORAL COMMUNICATIONS

SESSION 68: MANAGING FERTILITY PATIENTS EXPECTATIONS IN A PANDEMIC CONTEXT

01 July 2021 Stream 3 08:30 - 09:30

O-193 A devastating double disruption: IVF patients' feelings and experiences regarding treatment delays due to Covid-19

Z. Gurtin[1]

[1]*University College London, Institute for Women's Health, London, United Kingdom*

Study question: How did patients experience the delays and disruptions to their fertility treatment that occurred as a result of the Covid-19 pandemic and resulting clinic closures?

Summary answer: Patients reported feeling 'powerless/helpless' (78.3%), 'frustrated' (59.3%), and 'anxious' (54.7%), and detailed why clinic closures were experienced as a devastating double disruption.

What is known already: Fertility patients found clinic closures and the disruption to their treatments stressful due to uncertainty and perceived threats to their goal of parenthood, and experienced an increase in anxiety and depression. However, paper goes far beyond the mostly quantitative data that has been published by analysing patients' detailed qualitative accounts of their feelings and experiences in their own words.

Study design, size, duration: A mixed-methods, anonymous, online questionnaire in English was live for 6 weeks between 19 May to 30 June 2020. All patients aged over 18, whose fertility treatment or investigations had been impacted by the coronavirus pandemic were eligible to take part. The questionnaire was widely distributed using mainstream media, social media, and the mailing lists of relevant organisations. In total 709 people began and 501 completed the questionnaire in the time available (70.7% completion rate).

Participants/materials, setting, methods: The questionnaire included ten parts with a mixture of quantitative and qualitative items. The responses of 457 female fertility patients who were resident in the UK were analysed. The average age was 34.6 (SD=4.9). The majority were in a heterosexual relationship (91.0%), white (90.6%), and had no children (87.1%). Descriptive and inferential statistics were used on quantitative data, and thematic analysis used for qualitative data.

Main results and the role of chance: Using insights from the sociology of reproduction, including how patients face and resolve "disruption" (Becker 1997), this paper presents qualitative accounts from fertility patients regarding their feelings, reactions and experiences regarding the Covid-19 pandemic and the resulting clinic closures. Respondents highlighted the intensity of their feelings,

noting that their lives seemed "paused", "stopped" or "thrown into a state of limbo", leaving them unable to move forward with crucial life plans. Moreover, many explained that clinics closures were not experienced simply as a disruption, but rather as an additional hurdle in what had already been a series of difficult disruptions to normalcy, including, in many cases, an unforeseen inability to conceive naturally, long waiting lists for fertility treatment, and treatment delays due to economic or other factors. The major themes to emerge from respondents' accounts were: lack of control; lack of support; and feelings of difference, isolation and being left out. In many ways, the Covid-19 related disruptions exacerbated and added to fertility patients' existing anxieties and frustrations. One respondent wrote, "IVF is one of the most stressful things you can go through. To then be in the middle of that during a global pandemic it makes it even more stressful."

Limitations, reasons for caution: Participants were self-selecting and reporting their feelings and reactions at one particular point in time. Only responses from 457 UK-residents were included in the analyses.

Wider implications of the findings: These findings show that patients attending fertility clinics need additional support and care during times of uncertainty and disruption, and that many regard their treatment as an essential medical service. We encourage governments and regulators to keep fertility clinics open whenever it is possible to safely do so.

Trial registration number: Not applicable

O-194 Insights from smartphone app based emotional tracking data on the impact of the Covid-19 pandemic on IVF patients.

Y. Cheong[1], I. Robertson[1], J. Boivin[2]

[1]University of Southampton, Human Development and Health, Southampton, United Kingdom ;
[2]Cardiff University, School of Psychology, Cardiff, United Kingdom

Study question: Is the emotional experience of patients during IVF different since the start of the global Covid-19 pandemic?

Summary answer: Tracking data since re-opening demonstrated patients lower positive challenge emotions but no significant change in harm, threat, or stress.

What is known already: Covid-19 caused widespread shutdown of fertility centres, including in the UK, when the HFEA mandated closure from March until May 2020. Research shows clinic closure and an uncertain future were a significant psychological burden for patients anticipating treatment. However, emotional experiences before, during and after closure have not yet been compared, which is the aim of the study.

Study design, size, duration: Retrospective single-centre analysis of anonymised emotional tracking data entered by 707 patients using the MediEmo smartphone app alongside their IVF cycle, from May 2017-September 2020.

MediEmo includes medication timeline/ notifications, coping tools and emotional tracking. Patients rate 2 questions daily in each emotion domain (challenge, threat, harm, e.g. 'I am feeling tense') on a 0-3 scale and indicate coping ability ('I am unable to cope with the stress I am experiencing') on a binary scale.

Participants/materials, setting, methods: Egg donor, recipient and fertility preservation cycles were excluded. First, mood scores were analysed by 2020 month of entry to capture the emotional impact of closure. Second, "Pre-Covid" (May 2017-Feb 2020) and "After Re-opening" (May 2020–Sept 2020) emotional experiences were compared, using student t-tests. Mean and standard deviation of scores in each mood domain entered on each cycle day were calculated, centred on luteal day 0/ egg collection, from cycle day -14 to +14.

Main results and the role of chance: Graphical presentation of emotional data by month clearly demonstrates the significant increase in threat, harm and stress emotions and reduced positive emotions experienced immediately prior to and during mandatory clinic closure. Of patients entering emotional data during closure in March/April 2020, 40% (14/35) stated they felt unable to cope with the stress they were currently experiencing.

From May 2020 after the clinic reopened, analysis of in-cycle emotional tracking data showed there are no significant differences in harm or threat emotion levels or numbers reporting intolerable stress during IVF, compared to cycles pre-pandemic (May17-Feb 2020). Patients undertaking IVF cycles since closure are logging lower challenge scores (confident, encouraged, positive, hopeful), demonstrating less optimism, particularly in the 'two-week wait' phase of the cycle. The mean (s.d) of challenge scores pre-Covid was 1.50 (1.07), compared to 1.38 (1.04) after re-opening, p=0.00085.

The women who had treatment cycles post re-opening from May 2020 onwards were older (33.4(5.2) vs 32.6(4.4)), which may reflect clinical treatment prioritisation decisions. There was no significant differences in number of eggs collected (mean(s.d) Pre-Covid 12.08 (8.0) vs After re-opening 11.83 (9.4), p=0.84) or live birth/ ongoing pregnancy rates for undelivered pregnancies (p=0.69) between the groups.

Limitations, reasons for caution: Emotional data was only available for those who chose to use MediEmo, entered emotional tracking data and who gave consent for use of their clinical data in research. As such, this analysis may not fully reflect all patients' experiences. Most of the available data were entered prior to the pandemic.

Wider implications of the findings: For Covid-19 safety reasons, patients currently have less in-person staff contact when undertaking IVF. The findings reassuringly suggest emotional wellbeing was not markedly different in most domains. However, daily ratings did show the emotional fall-out of clinic closures which for most threatened attainability of parenthood goals (e.g., less hope).

Trial registration number: Not applicable

O-195 The impact of the COVID-19 outbreak on psychological distress due to the cancellation of ART. A systematic review and meta-analysis

Z. Donarelli[1], G. Lo Coco[2], S. Gullo[2], V. Oieni[1], A. Volpes[3], A. Allegra[3]

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[3]ANDROS Day Surgery Clinic, Reproductive Medicine Unit, Palermo, Italy

Study question: Is there evidence that infertile patients have been more likely to experience distress during the COVID-19 outbreak with the consequent interruption of treatment plans?

Summary answer: High levels of psychological distress among infertile patients have been found during the COVID-19 pandemic, greater than that reported in the general population.

What is known already: Preliminary research on the negative consequences of the COVID-19 outbreak on mental health evidenced heightened levels of anxiety, depression and post-traumatic stress in some clinical populations as well as in community samples. However, little is known about the impact of COVID-19 on psychological distress of infertile patients who have been forced to suspend infertility treatment and postpone parenthood goals during the pandemic. The aim of this meta-analytic review is to summarize extant literature on the prevalence of psychological distress symptoms in infertile patients during the COVID-19 pandemic.

Study design, size, duration: A systematic review and meta-analysis were conducted following the PRISMA guidelines on PsycInfo, PubMed, Embase, Web of Science, MedRxiv from March 2020 to mid-December 2020. Study inclusion criteria were specified according to the PICOS guideline. All naturalistic or RCT studies published in 2020 that examined infertility as the primary diagnosis and had a quantitative measurement of distress, were eligible. The primary outcomes were symptoms of psychological distress and secondary outcomes were indicators of psychological health.

Participants/materials, setting, methods: The database search identified 144 papers. Two reviewers independently screened potential studies by title and abstracts based on the inclusion criteria. The full texts were then screened for eligibility. The Newcastle-Ottawa Scale was used to judge the methodological quality of the studies. In order to estimate the pooled prevalence of distress, Odds Ratios with 95% Confidence Interval were calculated as the effect size by using a random-effects model. Heterogeneity was tested using I^2 statistics.

Main results and the role of chance: Fourteen studies met the inclusion criteria and were summarized for the systematic review (N=6473). Only six studies did not include males although, in the surveys, females made up 92% of the total sample. Ten studies adopted a cross-sectional study design. 100% gathered data through an online survey. Nine studies showed a high risk of bias, and five had a moderate risk. Review results showed that 56,4% of patients wished to resume treatment; participants were mostly worried about the delay in treatment because of their age (>35 years) or diminished ovarian reserve, or money

constraints and low education level. Only five studies examined the role of protective factors such as social support, coping, optimism trait and intolerance of uncertainty. Nine studies were included for meta-analysis. The prevalence of psychological distress was 0.58 (95% CI 0.32÷0.84). The pooled point estimates of prevalence for anxiety (N=6) were 0.56 (95% CI 0.24÷0.88), whereas the prevalence for depression (N=5) was 0.46 (95% CI 0.15÷0.77). There was significant heterogeneity among studies to estimate the prevalence (I^2 ranging from 99% to 100%).

Limitations, reasons for caution: Results are preliminary, given the small number of studies and their cross-sectional data.

The risk of bias was high or moderate across studies.

Wider implications of the findings: Infertile couples reported high levels of distress due to cancellation of their diagnostic procedures or treatment; they would benefit from information, appropriate support and advice from healthcare professionals, with an important role in maintaining the wishes of infertile couples to continue their parenthood goals.

Trial registration number: not applicable

O-196 The impact of providing couples with their IVF-prognosis on the expectations and anxiety of women and men.

J. Devroe[1,2], K. Peeraer[1,2], T. D'Hooghe[3,4,5], J. Boivin[6], J. Vriens[2], E. Dancet[2]

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[5]KU Leuven, Department of Development and Regeneration, Leuven, Belgium ;
[6]Cardiff University, School of Psychology, Cardiff, United Kingdom

Study question: What is the impact of providing couples with their IVF-prognosis on expectations and anxiety in women and men on the day of embryo transfer?

Summary answer: Only couples with a less than average IVF-prognosis updated their high expectations and IVF-prognosis was negatively associated with anxiety, especially in women.

What is known already: Female IVF-patients are known to expect a pregnancy rate per IVF-cycle of no less than 49-55%. Qualitative interviews and a survey showed that well informed women expect unrealistically high pregnancy rates as they think that their (family's) fertility and their clinic is better than average. Several prognostic models have recently been published. The adapted van Loendersloot model including clinical and laboratory characteristics proved performant for our clinic (AUC=0.74) and was validated internally (Devroe et al, BMJ Open, 2020). The impact of providing couples with their IVF-prognosis on expectations and wellbeing of female and male patients has yet to be studied.

Study design, size, duration: A prospective survey, questioning a final sample of 148 partnered individuals, completing their 2nd-6th IVF-cycle (2019-2020) in a University clinic, on the days of oocyte aspiration (OA) and fresh embryo transfer (ET). Thirty other partnered individuals declined participation (participation rate=85%) and 26 were excluded due to ET-cancellation. The IVF-prognosis (live birth rate, LBR, per completed IVF-cycle including fresh and frozen ETs from the same ovarian stimulation) was calculated with the adapted van Loendersloot model.

Participants/materials, setting, methods: Each partner reported their perception of their expected IVF-LBR on a visual analogue scale on the day OA. After being informed on their IVF-prognosis by gynaecologists, they re-rated their expected IVF-LBR and filled out the reliable 'STAI-State-Anxiety Inventory' on the day of fresh ET. Linear mixed models, taking account of partnering and assessing the association with gender, explored whether individuals updated their expected IVF-LBR after receiving their IVF-prognosis and whether IVF-prognosis and anxiety were associated.

Main results and the role of chance: The mean IVF-prognosis was 30.9% (±16.8). The 148 partnered individuals had a mean expected IVF-LBR of 59.1% (±20.0) on the day of OA (no gender effect; p=0.079). After being informed on their IVF-prognosis (day of ET), women's and men's mean expected IVF-LBR was 50.9% (±24.5) and 58.1% (±22.1), respectively (gender effect; p=0.002). Linear mixed models, including couple and time as random factors, did not show an effect of time on expected IVF-LBRs (p=0.15). Although women were more likely than men to update their expected IVF-LBR (p=0.002), the updates were not significantly different from the IVF-LBR expected on the day of OA (p=0.10). Women were more anxious than men (41.5±10.6 and 21.9±7.2, respectively, p<0.001) after being given their IVF-prognosis. Linear mixed models, including couple as a random factor, showed an association between IVF-prognosis and anxiety (p=0.016), especially in women (gender effect; p=0.004). Subgroup analysis showed that partnered individuals with lower than average prognoses (n=78) did update their expected IVF-LBR (p=0.036) while others (n=70) did not update their expected IVF-LBR (p=0.761). Among the subgroup with lower prognoses women were more likely to update their expected IVF-LBR than men (p=0.013), while no gender effect was observed among the subgroup with higher IVF-prognoses (p=0.078).

Limitations, reasons for caution: This is an explorative study in preparation of an adequately powered randomized controlled trial, testing whether couples who are informed on their IVF-prognosis update their expected IVF-LBR and whether this causes anxiety, as compared to care as usual in which couples are not informed on their IVF-prognosis.

Wider implications of the findings: Men and especially women with a less than average prognosis update their IVF-expectations after having received this prognosis which may trigger anxious reactions. These findings should be re-examined in an RCT. Following up the effect of sharing IVF-prognoses on longer-term distress and IVF-discontinuation would be interesting.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 69: EARLY PREGNANCY - EVIDENCE AND IMPLEMENTATION INTO PRACTICE

01 July 2021 Stream 4 08:30 - 09:30

O-197 Maternal and neonatal characteristics and outcomes of COVID-19 from early pregnancy until labor: an overview of systematic reviews

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Study question: What is the current obstetric-perinatal and neonatal outcome of infected pregnant women and their newborns during the COVID-19 pandemic?

Summary answer: Miscarriage rates were <2.5%, even when only studies of moderate/high-quality were included. Increased rates of CS and preterm birth were found, with uncertain vertical transmission.

What is known already: A considerable number of systematic reviews, with substantial heterogeneity regarding their methods and included populations, on the impact of COVID-19 on infected pregnant women and their neonates, has emerged.

Study design, size, duration: Three bibliographical databases were searched (last search: September 10, 2020). Quality assessment was performed using the AMSTAR-2 tool. Primary outcomes included mode of delivery, preterm delivery/labor, premature rupture of membranes (PROM/pPROM) and abortions/miscarriages. Outcomes were mainly presented as ranges. A separate analysis, including only moderate and high-quality systematic reviews, was also conducted. The protocol was registered with PROSPERO (CRD42020214447);

Participants/materials, setting, methods: The search strategy followed the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guideline. Keywords employed were (COVID-19 OR SARS-CoV-2 OR "Coronavirus disease 2019") AND ("Neonatal outcom*" OR "Neonatal

characteristic*" OR "Maternal outcom*" OR "maternal characteristic*" OR "pregnancy outcom*" OR "vertical transmission"). All retrieved studies were imported into the Rayyan QCRI and duplicated articles were removed. A snowball procedure was also implemented by hand-searching the reference lists of included systematic reviews for additional sources.

Main results and the role of chance: Thirty-nine reviews were analyzed. Twelve reviews (30.8%) were found to be of "very low quality", 11 of "low quality", 13 (33.3%) of "moderate", and three (7.7%) of "high quality".

Ten articles dealt with miscarriages. One review integrated them into pregnancy terminations (1.4% (4/295)), one into intrauterine fetal deaths (1(3%)), while another one described them as "spontaneous abortions" (0.8% (3/385)). Taking into account reviews, which calculated these rates for their entire included population, miscarriage rates were <2.5%. The reported rates by moderate and high-quality studies were ≤ 2%. Reported rates,regarding both preterm and term gestations, varied between 52.3%-95.8% for caesarean sections; 4.2%-44.7% for vaginal deliveries; 14.3%-63.8% specifically for preterm deliveries and 22.7%-32.2% for preterm labor; 5.3%-12.7% for PROM and 6.4%-16.1% for pPROM. Maternal anxiety for potential fetal infection contributed to abortion decisions, while SARS-CoV-2-related miscarriages could not be excluded. Maternal ICU admission and mechanical ventilation rates were 3%-28.5% and 1.4%-12%, respectively. Maternal mortality rate was <2%, while stillbirth, neonatal ICU admission and mortality rates were <2.5%, 3.1%-76.9% and <3%, respectively. Neonatal PCR positivity rates ranged between 1.6% and 10%. After accounting for quality of studies, ranges of our primary outcomes remained unchanged.

Limitations, reasons for caution: Results are presented in a narrative way using ranges as the primary mean of quantification. We also included studies with both RT-PCR positive women and women with suspected infection based on their clinical and imaging manifestations, whereas, if excluding them, we might have missed a considerable source of information.

Wider implications of the findings: In conclusion, a rapid increase of CS was observed, especially at the beginning of the pandemic, most likely due to lack of knowledge and robust recommendations. Preterm birth rates were elevated, with iatrogenic reasons potentially involved. Even though neonatal infections were rare, the probability of vertical transmission cannot be eliminated.

Trial registration number: not applicable'

O-198 Progesterone supplementation in women with threatened miscarriage: A randomised placebo-controlled clinical trial

L. McLindon[1], G. James[2], M.M. Beckmann[2], J. Bertolone[3], K. Mahomed[4], M. Vane[1], T. Baker[1], M. Gleed[1], S. Grey[1], L. Tettamanzi[1], B.W. Mol[5], W. Li[6]

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[6]Monash University, Obstetrics and Gynaecology, Melbourne, Australia

Study question: In women with threatened miscarriage, does progesterone supplementation increase the probability of live birth?

Summary answer: In women with threatened miscarriage, 400 mg progesterone nightly, from onset of bleeding until 12 weeks, did not increase live birth rates.

What is known already: Women with a history of miscarriage who present with bleeding in early pregnancy may benefit from the use of vaginal micronized progesterone 400 mg. A recently published large randomised clinical trial indicated no overall benefit for progesterone until 16 weeks, although subgroup analysis in women with bleeding and at least one previous miscarriage, progesterone might be of benefit (Coomarasamy et al; N Engl J Med 2019;380:1815-1824).

Study design, size, duration: We performed a single centre placebo-controlled randomised clinical trial. After informed consent, women with threatened miscarriage as apparent from vaginal bleeding under 10 weeks, were randomised to 400 mg vaginal micronized progesterone or placebo. The primary endpoint was livebirth. Secondary endpoints were perinatal outcomes, including preterm birth and birthweight. The planned sample size was 386 women. At a planned interim analysis randomisation was halted at 278 women due to lack of effectiveness and slow recruitment.

Participants/materials, setting, methods: Between February 2012 and April 2019 we randomised 139 women to 400 mg vaginal micronized progesterone and 139 women to placebo. Primary outcome data are available for 134 women in the progesterone arm and 130 women in the placebo arm. Mean age was 30.7 and 30.4 years. The number of women without a previous miscarriage was 68 (51%) and 55 (42%), while 66 (49%) and 75 (58%) women had at least one previous miscarriage.

Main results and the role of chance: The live birth rates were 113/134 (84.3%) and 112/130 (86.2%), respectively (RR 0.98, 95% CI 0.89-1.08). Among women with at least 1 miscarriage live birth rates were 55/66 (83.3%) and 65/75 (86.7%) (RR 0.96, 95% CI 0.84-1.11). The number of women with more than 1 miscarriage was limited (26 vs 33 in total), but no effect was seen from progesterone in these women. Preterm birth rates were 12.9% and 9.3% (RR 1.38; 95% CI 0.69 to 2.78). There were five pregnancy losses between 20 and 23 weeks, all in the progesterone arm. Mean birth weight was 3310 vs 3300 gram (p=.99). There were also no other differences in obstetric and perinatal outcomes. Anxiety, stress and depression scores did not differ between the groups.

Limitations, reasons for caution: Our study was single centre and did not reach the planned sample size. We stopped study medication at 12 weeks which might explain the difference between our study and studies that continued progesterone till 16 weeks.

Wider implications of the findings: In women with threatened miscarriage, 400 mg vaginal progesterone did not improve live birth rates.

Trial registration number: ACTRN12611000405910

O-199 Proof of concept: implantation window must be wider than proposed. Report of seven twins after asynchronous double embryo transfer

A. Gosálvez Vega[1], M. Rodriguez Mazaira[1], N. Martin Fernandez[1], M. Iglesias Nuñez[1], M. Brandt[1], L. Vidal Juan[1], P. Pastor Vargas[1], Z. Gonzalez Rodriguez[1], S. Corral Bermudez[1], R. García-Abadillo Seivane[1], R. Sainz de la Cuesta Abbad[2]

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Study question: Can simultaneous transfer of two embryos that were cryopreserved at different stages (D3 and Blastocyst) be appropriate to enhance success in women with more than three failed embryo transfers?

Summary answer: Double asynchronous embryo transfer offered excellent results in RIF. Unexpectedly high twin rate suggests that embryo-endometrium synchrony is overemphasized. Implantation window must be wider.

What is known already: Transcriptomic signature of the endometrium has been investigated in the last few years trying to understand the best moment for embryo implantation. Nevertheless, the optimal period has not been well established yet in humans. Simultaneous transfer of two human embryos at different developmental stages (D3 and Blastocyst) on Day 4 was proposed to help couples who have had RIF.

Study design, size, duration: Observational case-control study. From April 2016 to January 2021, we offered double asynchronous embryo transfer only after Recurrent Implantation Failure (RIF). Two requirements were necessary: 1) Double embryo transfer was acceptable by the couple due to poor reproductive outcome. 2) Availability of two embryos cryopreserved at different stage (D3 and Blastocyst). Results were compared with good prognosis patients (all patients under 35 years in that period who had elected to transfer two day 3 cryopreserved embryos).

Participants/materials, setting, methods: Forty-five patients accepted to participate in the study. Results were compared with all patients (237) under 35 years where two D3 thawed embryos were transferred. All cases received same protocol (oral estradiol 6mg/d or vaginal estradiol 4mg/d until ultrasound showed endometrial growth) LH, P4 and E2 were monitored in all patients to detect spontaneous LH surge. All cases received transvaginal micronized progesterone 800 mg/d. Embryo transfers were ultrasound guided and Wallace Embryosure catheter was employed.

Main results and the role of chance:

	Asynchronic	women < 35 years	Pearson's chi-squared test
Type of thawed embryos	D3 + Blastocyst in Prog. Day 4	two D3 in Prog. Day 3	
Transfers	51	237	
Embryos (n)	102	474	
Non pregnant (n) %	17 / 51 (33%)	61 / 237 (26%)	
Biochemical (n) %	3 / 51 (9%)	14 / 237 (8%)	
Clinical pregnancies (n) %	31 / 51 **(61%)**	162 / 237 **(68%)**	0,77 Probab. assoc. <90% (1)
Miscarriage/Ectopic (n) %	3 / 31 (10%)	16 / 162 (10%)	
Single ongoing preg. (n) %	21 / 28 **(75%)**	153 / 202 **(76%)**	
Twin ongoing preg. (n) %	7 / 28 (25%)	49 / 202 (24%)	0,03 Probab. assoc. <90% (2)

(1) Pregnancy rate was similar between groups, showing that double asynchronous transfer can be a good tool in some cases of RIF.
(2) Unexpectedly, simultaneous implantation of two embryos transferred with different progesterone timing wass possible. Even more, our results showed that multiple pregnancy rate was comparable to good prognosis patients prepared with optimal progesterone timing.

Limitations, reasons for caution: Multiple pregnancy rate was unacceptably high. Therefore, it should not be suggested for good prognosis couples where single embryo transfer is clearly advised. Our main limitation was the combination of D3 embryos with blastocysts. The retrospective design make the results to be considered as a proof of concept.
Wider implications of the findings: Double asynchronic embryo transfer can offer new insights in the understanding of human implantation. The concept of implantation window is clearly challenged. Aiming to the center of the window is fine, but we still don´t know how wide is that center.
Trial registration number: not applicable

O-200 Frozen embryo transfer: Miscarriage rates depending on the starting day of intramuscular progesterone.

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Study question: Objective was to assess whether adjusting starting day of intramuscular progesterone the day of vaginal supplementation versus day of embryo transfer or later, might affect the outcome of the cycle.
Summary answer: additional injection of intramuscular progesterone the day of progesterone initiation or later, is not likely to be more effective on live birth and miscarriage rates.
What is known already: There is no consensus on the most effective method of endometrium preparation prior to FET. However, many studies report that high serum progesterone concentration during the implantation period is associated with optimal live birth rates. Adjusting progesterone treatment the day of embryo transfer seems to be too late and ineffective for rescuing low progesterone levels and should be done before.
Study design, size, duration: In this single center prospective study from October 2019 to november 2020, 239 patients undergoing hormonal replacement therapy protocol for frozen embryo transfer were randomly divided into two groups: additional injection of intramuscular progesterone the day of progesterone initiation or intramuscular progesterone the day of embryo transfer. We compare these results to our previous protocol beginning intramuscular progesterone day 22 of the treatment.
Participants/materials, setting, methods: Our frozen embryo transfer protocol consists to initiate GnRH agonist the day 1 of the cycle. After 14 days of estrogens , we introduce vaginal progesterone , prior to embryo transfer. Patients in group A received an additional injection of intramuscular progesterone the day of progesterone initiation. The group B received intramuscular progesterone the day of embryo transfer. For both , intramuscular injection of progesterone was followed every 3 days.

Main results and the role of chance: 239 patients were enrolled in this study, 125 in the group A and 114 in the group B. The ongoing pregnancy rate in the group A was 26.4 % and miscarriage rate 7.2%, not statistically different from ongoing pregnancy rate and miscarriage rate of women in the group B (22.81 %, p= 0.66/ 6.14%, p= 0.8).

The ongoing pregnancy rate in the group D22 was 24.89 % et miscarriage rate 7.2%, not statistically different from ongoing pregnancy rate of women in the group A and B (p= 0.78 and p= 0.31).

	GROUP A	GROUP B	
Miscarriage (%)	7.2	6.14	NS
Ongoing pregnancies (%)	26.4	22.81	NS
Age (median age)	34.95	34.72	
Blastocyst (%)	91.49	90.90	

Limitations, reasons for caution: The main limitation of our study is the lack of randomization for the group with additional progesterone IM on day 22. The study is actually followed to enroll more patients in 3 different groups.
Wider implications of the findings: This study tries to determine optimal adaptive management of hormonal replacement treatment for embryo transfer in patients with potential low progesterone values.
Trial registration number: no applicable

SESSION 70: LIVE JOURNAL CLUB
01 July 2021 Stream 1 10:00-11:30

SELECTED ORAL COMMUNICATIONS
SESSION 71: ANEUPLOIDY AND MOSAIC ART
01 July 2021 Stream 2 10:00 - 11:30

O-201 Prenatal and postnatal outcome of mosaic embryo transfers: multicentric study of one thousand mosaic embryos diagnosed by preimplantation genetic testing with trophectoderm biopsy

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[6]Villa Mafalda, Reproductive Medicine, Rome, Italy ;
[7]Cooper Genomics-, Reproductive medicine, Livingston- New Jersey-, U.S.A.

Study question: To explore the effect of chromosomal mosaicism detected in preimplantation genetic testing (PGT-A) on prenatal and postnatal outcome of mosaic embryo pregnancies

Summary answer: No significant difference between euploid and mosaic embryos was observed in terms of weeks of gestation, average weight, and developmental defect of the babies born

What is known already: Mosaic embryos have the potential to implant and develop into healthy babies.Transfer of these embryos is now offered as an option for women who undergo IVF resulting in no euploid embryos. While, prenatal diagnosis has shown the depletion of chromosomal mosaicism in mosaic embryos, several concerns remain. For instance, the direct effects of different kind of mosaicism on prenatal/postnatal outcome and the possibility that intra-biopsy mosaicism in the TE is a poor predictor of the ploidy status of the ICM. Thus, there is certainly a need for comprehensive analyses of obstetrical and neonatal outcome data of transferred mosaic embryos.

Study design, size, duration: Compiled analysis from multicenter data on transfers of mosaic embryos (n=1,000) and their outcome, with comparison to a euploid control group (n=5,561). To explore the effect of embryonic mosaicism on newborns, we matched mosaic embryos resulting in a birth with a euploid embryo by a series of parameters (maternal age, embryo morphology, and indication for PGT-A). Prenatal tests and birth characteristics of >200 neonates from mosaic embryo transfers were compared to >200 euploid embryos.

Participants/materials, setting, methods: PGT-A was performed on blastocyst-stage embryos with 24-Chromosome whole genome amplification (WGA)-based Next Generation Sequencing (NGS). In accordance with established guidelines, embryos were categorized as mosaic when PGT-A results indicated 20-80% aneuploid content. Prenatal testing where performed in 30% of pregnancies with amniocentesis, 4% did an extra analysis for potential UPD for the suspected mosaic chromosome, and an additional 16% performed chorionic villus sampling (CVS) and 9.5% performed noninvasive prenatal testing (NIPT).

Main results and the role of chance: Of the 465 mosaic embryos that implanted, about 20% miscarried, and out of those, 75% were early spontaneous abortions. Of the pregnancies, 3 out of 368 were stillborn (2 out of them were twins that were extremely premature at 23 weeks, and the other died during pregnancy from a heart defect). The remaining 99% of those have been born or are late ongoing pregnancies at the time of analysis. Prenatal tests were performed in >200 pregnancies and the vast majority tested normal. All 5 abnormal cases were amniocentesis tests showing microdeletions or insertions of sizes smaller than the resolution used during PGT-A, so they were unrelated to the mosaicism detected with PGT-A. In fact, in none of the cases did the prenatal test reflect the mosaicism detected at the embryonic stage. Matching each of the 162 mosaic embryos resulting in a birth with a euploid embryo, we found that the length of gestation was similar on average, and so was the average weight of the babies at birth. We also gathered information on the routine physical examination performed on babies at birth, and of those 162 babies from mosaic embryo transfers, none had obvious developmental defects or gross abnormalities.

Limitations, reasons for caution: Even though newborns resulting from mosaic embryo transfers in this study invariably appeared healthy by routine examination, concerns for long-term health cannot yet be entirely dispelled. The question must therefore be carefully considered by each clinic and patient situation.

Wider implications of the findings: Prenatal testing of >200 pregnancies from mosaic embryo transfers showed no incidence of mosaicism that matched the PGT-A findings, indicating the involvement of self-corrective mechanisms. Pregnancy and obstetric data indicates that mosaic embryos prevailing through gestation and birth have similar chromosomal and physiological health compared to euploid embryos.

Trial registration number: none

O-202 Usage of laser during trophectoderm biopsy and its affect on PGT-A results and moscaism

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Study question: Does laser use during trophectoderm biopsy affect biopsy results on prehatching embryos with regard to mosaicism ?

Summary answer: According to our findings laser usage during trophectoderm biopsy increases mosaic results on next generation sequencing (NGS) irrespective of embryo quality.

What is known already: Chromosomal mosaicism, which is a result of mitotic errors after fertilization, is defined as the presence of karyotypically distinct cell lines within an embryo. The introduction of NGS made it possible to detect chromosomal mosaicism at levels as low as 20%. The incidence of mosaicism is highly variable between clinics which reported the incidences between 4-32%. Apart from the biological reasons, there are also various technical factors that may impact the incidence of mosaicism. One of the most emphasized factors is the trophoectoderm biopsy technique. Laser usage and number of laser pulses may cause excessive heat during the procedure

Study design, size, duration: The mosaicism ratio in embryos in which trophectoderm biopsy was performed with or without laser, between January 2017 - December 2020 in Istanbul Memorial Hospital (IMH) were examined retrospectively. A total of 13002 embryos were analyzed. A subgroup analysis was also performed regarding mosaicism ratios in different embryo qualities. Blastocysts were classified according to Gardner's classification and classified as follows: top quality-TQ (4AA,5AA,6AA), good quality-GQ (3AA, 4,5,6AB,BA) and moderate quality-MQ (3,4,5 BB).

Participants/materials, setting, methods: The biopsy samples of the cases who had PGT-A in IMH between 2017-2020 were evaluated by NGS method. This method enables the identification of embryos with 20% to 80% mosaicism. The study assessed whether there was an increase in the embryos with mosaic results due to the use of laser during biopsy. The effects of laser use among the TQ (4AA,5AA,6AA), GQ (3AA, 4,5,6AB,BA) and MQ (3,4,5 BB) groups according to Garder classification were analyzed.

Main results and the role of chance: Trophectoderm biopsy was applied on 13002 embryos within the specified period. During biopsy in 5088 embryos laser was used and in 7843 embryos laser was not used, and biopsy was performed mechanically (flicking method). After observing the biopsy results, 945/5088 (18.5%) of the laser applied embryos; and 1087/7914 (13.7%) of laser not applied embryos were defined as mosaic($p < 0.0001$). When mosaicism rates were examined according to embryo qualities, the rate of mosaicism was 19.3%(469/2430), 18.2%(290/1591) and 13.2%(380/2875), 13.5 (426/3141) respectively in embryos with and without laser in TQ and GQ groups. A statistically high level of significance ($p < 0.0001$) was observed between the embryos evaluated as top quality and good quality before biopsy. Regarding the evaluation in the moderate group embryos, although the mosaicism rates tended to increase on the laser applied group side 40/248(16.1%), no statistical difference was observed when compared to non-laser group 103/670(15,4%). ($P > 0.05$)

Limitations, reasons for caution: The retrospective nature of the data is the main limitation of the study. On the other hand, the large number of NGS based PGT-A tested TQ and GQ embryos from a single center and resuts from single laboratory.However, further studies are required to corroborate our findings.

Wider implications of the findings: Laser dependent heat effect may increase mosaicism. To reduce the cell damage, teasing of cells should be avoided and a minimum number of laser pulses should be used in order to avoid excessive heat and contact points should be preferably confined to cell junctions

Trial registration number: None

O-203 Application of machine learning to predict aneuploidy and mosaicism in embryos from in vitro fertilization (IVF) cycles

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Study question: Is it possible to predict the likelihood of an IVF embryo being aneuploid and/or mosaic using a machine learning algorithm?
Summary answer: There are paternal, maternal, embryonic and IVF-cycle factors that are associated with embryonic chromosomal status that can be used as predictors in machine learning models.
What is known already: The factors associated with embryonic aneuploidy have been extensively studied. Mostly maternal age and to a lesser extent male factor and ovarian stimulation have been related to the occurrence of chromosomal alterations in the embryo.

On the other hand, the main factors that may increase the incidence of embryo mosaicism have not yet been established.

The models obtained using classical statistical methods to predict embryonic aneuploidy and mosaicism are not of high reliability. As an alternative to traditional methods, different machine and deep learning algorithms are being used to generate predictive models in different areas of medicine, including human reproduction.
Study design, size, duration: The study design is observational and retrospective. A total of 4654 embryos from 1558 PGT-A cycles were included (January-2017 to December-2020). The trophoectoderm biopsies on D5, D6 or D7 blastocysts were analysed by NGS. Embryos with ≤25% aneuploid cells were considered euploid, between 25-50% were classified as mosaic and aneuploid with >50%.

The variables of the PGT-A were recorded in a database from which predictive models of embryonic aneuploidy and mosaicism were developed.
Participants/materials, setting, methods: The main indications for PGT-A were advanced maternal age, abnormal sperm FISH and recurrent miscarriage or implantation failure. Embryo analysis were performed using Veriseq-NGS (Illumina).

The software used to carry out all the analysis was R (RStudio). The library used to implement the different algorithms was caret. In the machine learning models, 22 predictor variables were introduced, which can be classified into 4 categories: maternal, paternal, embryonic and those specific to the IVF cycle.
Main results and the role of chance: The different couple, embryo and stimulation cycle variables were recorded in a database (22 predictor variables). Two different predictive models were performed, one for aneuploidy and the other for mosaicism. The predictor variable was of multi-class type since it included the segmental and whole chromosome alteration categories.

The dataframe were first preprocessed and the different classes to be predicted were balanced. A 80% of the data were used for training the model and 20% were reserved for further testing. The classification algorithms applied include multinomial regression, neural networks, support vector machines, neighborhood-based methods, classification trees, gradient boosting, ensemble methods, Bayesian and discriminant analysis-based methods. The algorithms were optimized by minimizing the Log_Loss that measures accuracy but penalizing misclassifications.

The best predictive models were achieved with the XG-Boost and random forest algorithms. The AUC of the predictive model for aneuploidy was 80.8% (Log_Loss: 1.028) and for mosaicism 84.1% (Log_Loss: 0.929). The best predictor variables of the models were maternal age, embryo quality, day of biopsy and whether or not the couple had a history of pregnancies with chromosomopathies. The male factor only played a relevant role in the mosaicism model but not in the aneuploidy model.
Limitations, reasons for caution: Although the predictive models obtained can be very useful to know the probabilities of achieving euploid embryos in an IVF cycle, increasing the sample size and including additional variables could improve the models and thus increase their predictive capacity.
Wider implications of the findings: Machine learning can be a very useful tool in reproductive medicine since it can allow the determination of factors associated with embryonic aneuploidies and mosaicism in order to establish a predictive model for both. To identify couples at risk of embryo aneuploidy/mosaicism could benefit them of the use of PGT-A.
Trial registration number: Not Applicable

O-204 Is mosaicism affected by an embryologist's experience in biopsy?

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Study question: Are there any correlations between blastocyst mosaicism rate and biopsy experience among embryologists?
Summary answer: Blastocysts biopsied by embryologists with ≥1 year of biopsy experience have significantly lower mosaicism rate compared to those with <1 year of biopsy experience.
What is known already: It has been reported that the incidence of blastocyst mosaicism is highly variable between centres (PGDIS, 2019). It is also suggested that the technical aptitude of the embryologist performing blastocyst biopsy may give rise to mosaicism. Thus, a retrospective study was conducted to investigate the relationship between blastocyst mosaicism rate and biopsy experience among embryologists in Alpha IVF.
Study design, size, duration: Thirteen competent embryologists who were trained in blastocyst biopsy were included in this study: 5 have ≥1 year of biopsy experience (Group A; Embryologist A-1, A-2, A-3, A-4, A-5); 8 have <1 year of biopsy experience (Group B; Embryologist B-1, B-2, B-3, B-4, B-5, B-6, B-7, B-8). Embryologists from Group A biopsied a total of 4795 blastocysts while those from Group B biopsied 4869 blastocysts from January 2018 to December 2019.
Participants/materials, setting, methods: TE biopsy was performed either on Day 5, 6 or 7 using the laser or flicking method. The biopsied cells had Preimplantation Genetic Testing for Aneuploidy (PGT-A) analysed using Next Generation Sequencing (Ion Torrent, USA) and chromosomal mosaicism analysis was done using ReproSeq Mosaic PGS w1.1 workflow. Mosaic blastocysts were reported when 20% - 80% of aneuploid cells are tested in the biopsied samples. Only successfully amplified biopsy samples were included in this study.
Main results and the role of chance: The mosaicism rate of blastocysts biopsied by embryologists from Group A and B were 17.8% and 19.8% respectively. Blastocysts from Group A showed significantly lower mosaicism rate compared to Group B ($p=0.01$). The mosaicism rates of blastocyst biopsied by Embryologist A-1, A-2, A-3, A-4 and A-5 were 17.3%, 19.1%, 16.8%, 15.2%, and 18.9% respectively. The mosaicism rates of blastocyst biopsied by Embryologist B-1, B-2, B-3, B-4, B-5, B-6, B-7, and B-8 were 17.5%, 18.6%, 22.5%, 20.4%, 27.8%, 20.6%, 20.1% and 20.3% respectively. There were no significant differences in blastocyst mosaicism rate between embryologists within Group A ($p>0.05$). Contrarily, in Group B, Embryologist B-5 had a significantly higher blastocyst mosaicism rate compared to the other embryologists within the same group ($p<0.05$).
Limitations, reasons for caution: Since this study is retrospective in nature, the biopsy technique (either the laser or flicking method) was not controlled. Hence, further studies to analyse the differences between these 2 biopsy techniques should be carried out to confirm its effect on the occurrence of blastocyst mosaicism.
Wider implications of the findings: Our study demonstrates that blastocysts biopsied by embryologists with ≥1 year of biopsy experience have significantly lower mosaicism rate compared to those with <1 year of biopsy experience. This indicates that the skill and experience of an embryologist in biopsy may have an impact on the mosaicism rate.
Trial registration number: Not applicable

O-205 Aneuploidy induces proteotoxic stress and autophagy-mediated apoptosis in human preimplantation embryos

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Study question: Are aneuploid cells in human preimplantation embryos eliminated by apoptosis due to proteotoxic stress and autophagy-mediated apoptosis?
Summary answer: Proteotoxic stress, autophagy and apoptosis are differentially activated in aneuploid embryos, showing that aneuploid cells are eliminated by these mechanisms during early human embryogenesis.
What is known already: Aneuploidies are a common feature of human preimplantation embryos which could explain low success rates after *in vitro* fertilization (IVF). While most aneuploidies of meiotic origin are detrimental, transfer of euploid-aneuploid mosaic embryos can lead to healthy live-births. Moreover, the proportion of aneuploid cells are lower in blastocysts when compared to cleavage stage embryos. In the mouse, aneuploid cells are eliminated from the epiblast by autophagy-mediated apoptosis in a p53-dependent manner. We propose that in human embryos, aneuploidy causes chronic protein misfolding which leads to autophagy-induced apoptosis.
Study design, size, duration: Eighty-one blastocysts that were diagnosed by PGT as euploid (n=49) or uniformly combined abnormal (CA, n=32), i.e. 2 or more chromosomes were abnormal in every cell, were warmed. Sixty-seven were suitable for trophectoderm (TE) biopsy, 54 biopsies were successfully tubed and sent for RNA-sequencing while the remainder of the embryos was fixed for immunostaining. Thirty-three day-3 embryos were overnight incubated in 0.5µM reversine allowed to develop into blastocysts and treated as the PGT embryos.
Participants/materials, setting, methods: After TE biopsy, we live-stained the embryos with either Caspase-3/7 or 8 and subsequently fixed them. The biopsies underwent RNA-sequencing using the SMART-seqv4 and the fixed embryos were immunostained for LC3B, p62 (autophagy) and HSP70 (proteotoxic stress). Confocal imaging was performed using a Zeiss LSM800 confocal microscope and the presence of signal was quantified using the Zen Blue 2.0 and Arivis software.
Main results and the role of chance: Forty-two percent of the embryos in which we induced aneuploidies using reversine developed into blastocysts, which is comparable to untreated embryos. After immunostaining, we observed that CA and reversine-treated (RT) embryos contained less cells than euploid embryos (median number of nuclei: 43.5, 47, 90, respectively). This correlates with a higher expression of apoptotic markers Caspase-3/7 in CA embryos (p=0.0199) and Caspase-8 in both aneuploid groups (CA: p=0.0085 and RT: p=0.0394). Aneuploid embryos showed significantly increased HSP70 levels (median intensity per cell: euploid=165, CA=313, RT=400), LC3B (median puncta per cell: euploid=3.07, CA=10.10, RT=19.62) and p62 (median puncta per cell: euploid=17.60, CA=30.53), suggesting increased proteotoxic stress and autophagy. Preliminary analysis of the RNA-sequencing data reveals enrichment for pathways such as the p53-pathway, protein secretion, TNFA signaling via NFkB and apoptosis, supporting the hypothesis of a link between aneuploidy and apoptosis.
Limitations, reasons for caution: No functional tests e.g. with inhibitors of autophagy were carried out. RNA-sequencing was carried out on a small sample; we will expand this sample in the near future.
Wider implications of the findings: This study shows for the first time the mechanism by which aneuploid cells are eliminated from the human preimplantation embryo, explaining how mosaic embryos can still lead to a healthy and genetically normal live birth.
Trial registration number: not applicable

O-206 Meiotic segregation analysis for reciprocal translocation carriers: Assessment of factors influencing meiotic segregation patterns

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Study question: To analyze factors that could influence meiotic segregation patterns for reciprocal translocation carriers.
Summary answer: Involvement of an Acr-ch, female gender, and lower TAR1 (ratio of translocated segment 1 over the chromosome arm) were independent risk factors for alternate segregation.
What is known already: Reciprocal translocation is one of the more common structural rearrangements of chromosomes, which is associated with reproductive risks, such as infertility, spontaneous abortion and the delivery of babies with mental retardation or developmental delay. Extensive studies on meiotic segregation patterns of sperm, blastomere, and blastocysts have identified several factors that may influence the generation of unbalanced rearrangement of reciprocal translocations, including carrier's gender and age, location of breakpoints, chromosome type, and the quadrivalent structure. However, some results are controversial.
Study design, size, duration: A retrospective study from October 2013 to December 2019, a total of 10846 blastocysts originating from 2871 oocyte retrieval cycles from 2253 couples with one of the partners carrying reciprocal were investigated. The mean maternal age was 29.97±4 years (20 –47years).
Participants/materials, setting, methods: Trophectoderm biopsy of blastocysts was performed on the 5th or 6th day of development. Whole genome amplification (WGA) was performed on all samples, and the WGA was analyzed with SNP array or NGS. Segregation patterns of quadrivalent in 10846 blastocysts were analyzed. Risk factors for segregation patterns were explored through analyzing carriers' demographic and cytogenetic characteristics using multivariate generalized linear mixed models (GLMMs).
Main results and the role of chance: The percentage of normal/balanced blastocysts was 34.3%, and 2:2 segregation was observed in 90.0% of blastocysts. Increased TAR1 (the ratio of translocated segment 1 over the chromosome arm) was noted as an independent protective factor for the proportion of alternate segregation (P=0.004). The female gender and involvement of an Acr-ch were found independent risk factors for alternate segregation (P<0.001). A higher TAR1 reduced the risk of adjacent-1 segregation; longer translocated segment and female gender increased the risk of adjacent-2 segregation (P=0.009 and P<0.001, respectively). Female gender and involvement of an Acr-ch enhanced the risk of 3:1 segregation (P<0.001 and P=0.012, respectively).
Limitations, reasons for caution: About 1400 blastocysts were not diagnosed in the 2871 cycles, which might cause bias in the results. Secondly, the interchromosomal effect of reciprocal translocations was not analyzed in this study.
Wider implications of the findings: In conclusion, a carrier's gender, involvement of an Acr-ch, and location of breakpoints may influence the segregation patterns. Besides, involvement of an Acr-ch, female gender, and lower TAR1 are independent risk factors for alternate segregation. These results may provide more appropriate genetic counseling for couples with balanced translocation.
Trial registration number: no

SELECTED ORAL COMMUNICATIONS

SESSION 72: SPERM DNA FRAGMENTATION: ALIVE AND KICKING

01 July 2021 Stream 3 10:00 - 11:30

O-207 Which infertile patients mostly deserve to have a sperm DNA fragmentation index done? Findings from a cross-sectional study

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Study question: Current scientific guidelines do not clearly suggest which patients would benefit the most from a sperm DNA fragmentation (SDF) test.
Summary answer: We aimed to investigate potential predictive factors for altered SDF in a homogenous cohort of white-European men presenting for primary couple's infertility.
What is known already: High SDF has been associated with reduced fertilization rates, reduced chances of natural conception and an increased risk of early pregnancy loss.
Study design, size, duration: Data from 478 consecutive men with normal or altered SDF were analysed. Infertility was defined according to the WHO criteria. Semen analysis, SDF (according to SCSA) and serum hormones were measured in every patient. Health significant comorbidities were scored with the Charlson Comorbidity Index (CCI). Altered SDF was considered with a threshold of >30%.
Participants/materials, setting, methods: Descriptive statistics compared the overall characteristics of patients with normal SDF and altered SDF. Logistic regression analysis tested potential predictors of altered SDF. ROC curve was used to test the accuracy of the model in predicting SDF alteration
Main results and the role of chance: Of 478 patients, 253 (57.7%) had altered SDF. Median (IQR) age and BMI of the whole cohort were 38 (35-42) years and 25.1 (23.3-27.1) kg/m^2 respectively. Patients with altered SDF were older (median (IQR) age: 39 (36-43) vs. 37 (34-38) years, p<0.0001), had lower sperm concentration (5 (1.1–18) vs. 17 x10^6/mL (6–38.8), p<0.0001), testicular volume (15.1 (12 –20) vs. 16.8 (12 – 25) Prader, p=0.0005), and total motile sperm count (TMSC) (1.8 (0.21–10.71) vs. 11.8x10^6 (2–37.26), p<0.0001). Conversely, men with altered SDF had higher FSH (6.1 (3.85–9.7) vs. 4.8 (3.85 – 7.9) mIU/mL, p<0.0001) and prolactin levels (9.8 (7.43–14.04) vs. 8.3 (6.6–11.3) pg/mL, p=0.0004) than those with normal SDF. At multivariable logistic regression analysis, patients' age >35 years (OR: 2.45, p=0.0009), FSH > 8.0 mIU/mL (OR: 2.23, p<0.0001) and lower TMSC (OR: 2.04, p=0.002) were identified as indipendent predictors of altered SDF, after adjusting for testicular volume and CCI≥1. ROC curve (Figure 1) revealed that the model has a good predictive ability to identify patients with SDF alteration (AUC: 0.72, 95%CI: 0.67 - 0.77).
Limitations, reasons for caution: It is a retrospective analysis at a single, tertiary-referral academic centre, thus raising the possibility of selection biases. In spite of this, all patients have been consistently analysed over time with a rigorous follow-up, thus limiting potential heterogeneity in terms of data reporting
Wider implications of the findings: Primary infertile men older than 35 years, with high serum FSH and low TMSC at baseline are the ones who mostly deserve a SDF test over their diagnostic work-up and that would potentially benefit the most of certain treatments to improve SDF value, thus increasing chances of conceiving.
Trial registration number: Not applicable

O-208 High sperm DNA fragmentation index negatively impacts embryo morphokinetics, but not embryo morphology and development rates: the importance of time-lapse imaging system

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Study question: Can time-lapse imaging (TLI) identify morphokinetic events impacted by high sperm DNA fragmentation index (DFI), irrespective of conventional morphological embryo assessment and development rate?
Summary answer: Embryo morphokinetic parameters are negatively impacted by high DFI, whereas conventional morphological embryo assessment and blastocyst development rate are not related to DNA integrity.
What is known already: Paternal genome activation occurs late in the embryo, and therefore, the negative impact of sperm factors on embryo development is more often observed in the outcomes of pregnancy, such as embryonic implantation and pregnancy loss, than in the potential for embryonic development itself, such as successful development to the blastocyst stage. With the development of TLI technology, and the possibility of assessing complete embryonic development, we hypothesized that sperm factors related to DNA fragmentation may interfere with the speed and pattern of cell divisions, leading to slower embryos; something that would not be detected by conventional morphological embryo assessment.
Study design, size, duration: The study included 978 zygotes cultured until day five in a TLS incubator between March/2019 and August/2020, derived from 118 patients undergoing ICSI in a private university-affiliated IVF center. Kinetic markers from the point of insemination were recorded. Generalized linear models adjusted for potential confounders, followed by Bonferroni post hoc were used to compare timing of specific events in patients with low (<30%) of high (≥30%) DFI. The post hoc achieved power was > 90%.
Participants/materials, setting, methods: Recorded kinetic markers were: timing to pronuclei appearance and fading (tPNa and tPNf), timing to two (t2), three (t3), four (t4), five (t5), six (t6), seven (t7), and eight cells (t8), and timing to morulae (tM), start of blastulation (tSB) and blastulation (tB). Durations of second and third cell cycles (cc2 and cc3) and timing to complete synchronous divisions s1, s2, and s3 were calculated. The KIDScore ranking was also recorded.
Main results and the role of chance: Blastocyst development (53.1% ± 1.3 vs. 55.1% ± 1.5, p=0.380) and high-quality rates (87.9% ± 2.9 vs. 86.2% ± 3.6, p=0.749) were similar between embryos derived from sperm samples with <30% DFI (n=592) and ≥30% DFI (n=386), respectively. Embryos derived from sperm samples with ≥30% DFI showed significantly slower divisions compared to those from <30% DFI: tPNa (6.1h ± 0.2 vs. 6.8h ± 0.2, p=0.030), tPNf (23.0h ± 0.3 vs. 24.2h ± 0.3, p=0.009), t2 (25.4h ± 0.3 vs. 26.9h ± 0.3, p=0.002), t3 (34.8h ± 0.3 vs. 37.3h ± 0.4, p>0.001), t4 (37.5h ± 0.4 vs. 39.3h ± 0.4, p=0.003), t5 (46.2h ± 0.5 vs. 49.5h ± 0.6, p<0.001) , t6 (49.7h ± 0.5 vs. 52.8h ± 0.6, p=0.001), t7 (52.4h ± 0.6 vs. 55.6h ± 0.7, p=0.001), t8 (56.2h ± 0.7 vs. 58.9h ± 0.8, p=0.017), tSB (97.5h ± 1.5 vs. 105.9h ± 1.7, p=0.002), tB (108.6h ± 0.8 vs. 112.4h ± 1.2, p=0.016). The KIDScore ranked significantly different between embryos derived from samples with <30% or ≥30% DFI (4.5 ± 0.1 vs. 3.9 ± 0.2, p=0.033, respectively). A significant difference was observed in implantation rate (<30% DFI: 51.5% ± 2.2 vs. ≥30% DFI: 30.5% ± 1.3, p<0.001).
Limitations, reasons for caution: Retrospective nature of this study and the small sample size may be a reason for caution, despite adequate power has been achieved.
Wider implications of the findings: Increasing DFI correlates with delayed cell cleavage and blastulation, leading to reduced implantation rates, without compromising blastulation rate and quality. This finding highlights the importance of TLI for the identification and de-selection of slow-growing embryos for transfer, in cycles with high DFI.
Trial registration number: Not applicable

O-209 The impact of sperm DNA fragmentation on pregnancy outcomes depends on oocyte dimorphisms

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Study question: Does the impact of sperm DNA fragmentation (SDF) on Intracytoplasmic sperm injection (ICSI) outcomes depend on the presence of oocyte dimorphisms?
Summary answer: There is a significant influence of oocyte quality on the impact of SDF on pregnancy outcomes.
What is known already: Sperm DNA fragmentation has been associated with ICSI outcomes. DNA damage is commonly encountered in human spermatozoa and it has been widely accepted that the oocyte assumes responsibility for the repair and remodelling of both the maternal and paternal genomes during the oocyte-embryo transition. Indeed, spermatozoa with DNA damage can fertilise oocytes and still lead to embryo development due to the oocyte DNA repair capacity. Considering the vital role played by the oocyte in the developmental process, it could be hypostatised that the oocyte quality, translated as oocyte morphology, would influence the machinery responsible for sperm DNA repair after fertilization.
Study design, size, duration: This cohort study included 3,035 oocytes from 525 patients undergoing ICSI cycles in a university-affiliated IVF-center, between June/2016 and July/2019. Oocytes were split into groups according to the SDF

index of the sample used for ICSI: low-fragmentation (<30% SDF, n= 2,277) and high-fragmentation (≥30% SDF, n=758). Oocytes were evaluated before sperm injection and the dimorphisms were recorded. The influence of SDF index on ICSI outcomes, depending on the presence of oocytes dimorphisms was evaluated.

Participants/materials, setting, methods: Data was evaluated using generalized linear models (GZLM) followed by Bonferroni post hoc. The results are expressed as mean ± standard error for continuous variables or percentages for dichotomous variables, and p-values. The sample size calculation suggested that a sample of at least 504 subjects had 95% power to detect a 20% effect with a significance level of 5% ( ). The study was performed in a private university–affiliated in vitro fertilization (IVF) center.

Main results and the role of chance: The association of both factors: the presence of oocyte dimorphisms (dark cytoplasm, vacuoles in the ooplasm, and resistant membrane) and high SDF index resulted in the lowest fertilization rate among groups, while oocytes free of these dimorphisms injected with samples with <30% SDF had the highest fertilization rate (p=0.05, p<0.01 and p <0.01 for dark cytoplasm, vacuoles in the ooplasm and resistant membrane respectively). The impact of SDF index on high quality embryos rate on cleavage stage was also influence by the presence smooth endoplasmic reticulum clusters and resistant membrane oocytes (p=0.013 and p= 0.018). As for the clinical outcomes, the impact of SDF index on the implantation rate was influenced by the presence of vacuoles in the ooplasm (p<0.01), smooth endoplasmic reticulum clusters (p<0.01), large perivitelline space (p<0.01), resistant membrane (p<0.01), and non-resistant membrane (p<0.01), while the influence of SDF index on the pregnancy rate was influenced by the presence large perivitelline space (p<0.01), resistant membrane (p=0.018) and non-resistant membrane (p<0.01). The effect of SDF on the miscarriage rate was also increased in the presence of large perivitelline space (p=0.045), non-resistant membrane (0.037) and centrally located cytoplasmic granular area (p=0.025).

Limitations, reasons for caution: The retrospective nature is a limitation. It could be argued that using samples with high SDF index does not necessarily mean that a sperm cell with a fragmented DNA was injected, however, the higher the SDF index, the higher the chance of selecting one with fragmented DNA.

Wider implications of the findings: The findings presented here highlight the crucial role of male and female factors when facing assisted reproduction. The association of low oocyte quality and high SDF index may lead to impaired results. As the oocyte defect cannot be modified, in vivo upgrading of spermatozoa before the treatment should be encouraged.

Trial registration number: Not applicable

O-210 Sperm DNA fragmentation (SDF) is not associated with adverse maternal and neonatal outcomes in IVF-ICSI cycles with autologous oocytes

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Study question: Does an elevated SDF (>15%) increase the odds of adverse maternal and neonatal outcomes in autologous oocyte IVF-ICSI cycles from unselected couples?

Summary answer: No adverse effects of high SDF on obstetric and neonatal outcomes have been found in couples with sperm fragmentation undergoing IVF-ICSI cycles with own eggs.

What is known already: Sperm chromatin integrity assessment has been implemented as an additional tool in the clinical evaluation of sperm quality in infertile patients undergoing an assisted reproduction treatment. All of the published reports to date appraise its effect on clinical outcomes, and how it impacts embryo quality and the pregnancy chances after IVF and ICSI cycles. Sperm DNA integrity has also been hypothesized to affect offspring health but not many studies have reported in humans if an elevated SDF raises the risks of obstetric, delivery and neonatal outcomes.

Study design, size, duration: Multicentric retrospective cohort study of all IVF-ICSI cycles using autologous oocytes between January 2000-March 2019 at Spain IVIRMA clinics of couples with a SDF test on their ejaculated semen. The sperm fragmentation index was measured in all men with TUNEL assay. The database included 228 couples which had a delivery with at least a newborn. Subjects were divided into two study groups according to their level of SDF: ≤15% (low SDF) or >15% (high SDF).

Participants/materials, setting, methods: Patients with missed information on maternal and neonatal outcomes were not counted for the analysis. The obstetric outcomes were gestational age, gestational diabetes, preeclampsia (hypertension with proteinuria after 20 weeks of gestation) and type of delivery. Neonatal outcomes were sex, birth weight, length, head circumference, Apgar score at 1, 5, 10 minutes, and neonatal intensive care unit (NICU) admission. Student's t-test and Fisher's test were used for statistical analysis. A *p-value*<0.05 was considered statistically significant.

Main results and the role of chance: Maternal age mean was 37.4 years (95%CI 36.9-38.0) in ≤15%SDF group and 37.2 years (95%CI 36.1-38.4) in >15%SDF group (p=0.8). Similar gestational age was found, 41.8 weeks (95%CI 41.3-42.2) in ≤15%SDF and 41.3 weeks (95%CI 40.4-42.3) in >15%SDF. Gestational diabetes incidence was higher in >15%SDF compared to ≤15%SDF group (3.5% versus 1.7% (OR=2.0 (95%CI 0.03-39.8), p=0.5). Equally, the incidence of preeclampsia was 3.6% in patients with high SDF versus 1.7% in couples with low SDF, OR=2.1 (95%CI 0.03-41.3), p=0.5. Type of delivery frequency was in the ≤15%SDF group 61.9% vaginal and 38.1% cesarean, while in the >15%SDF group 62.1% vaginal and 37.9% cesarean (OR=1.0 (95%CI 0.4-2.6), p=1.0). The overall proportion of singleton pregnancies was 87.2% (95%CI 82.4-91.2) and twins 12.8% (95%CI 8.8-17.6). There were no statistically differences between groups in the rate of delivery of twins and in the sex ratio of the newborns. When comparing the newborns of ≤15%SDF with >15%SDF group, the average of weight was 3011.7g (95%CI 2912.2-3111.2) versus 2986.4g (95%CI 2753.1-3219.7), of length was 49.2cm (95%CI 48.3-50.0) versus 49.5cm (95%CI 49.2-49.9), of head circumference was 34.9cm (95%CI 34.6-35.2) versus 34.3cm (95%CI 33.4-35.2). No statistically differences were observed for Apgar punctuation and for NICU admission.

Limitations, reasons for caution: Due to the retrospective nature of the study we have missing data from the lack of follow-up of many patients after the confirmation of the ongoing pregnancy. Although pregnancies of couples with elevated SDF have a higher incidence of gestational diabetes and preeclampsia, the sample size evaluated is a limitation.

Wider implications of the findings: This is one of the first reports to evaluate the relationship between paternal DNA damage and obstetric risks and neonatal health in couples with high SDF who underwent IVF-ICSI in our centers. Despite SDF did not jeopardize the maternal and neonatal outcomes, more studies are needed to confirm this conclusion.

Trial registration number: NA

O-211 The DNA double-strand break repair genes expression exhibits significant changes in the postnatal mouse testes from early to aged terms

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Study question: How does the DNA double-strand break (DSB) repair genes, *Rad51*, *Rpa70*, *Ku80*, and *Xrcc4*, expression change in the postnatal mouse testes from early to aged terms.

Summary answer: The *Rad51*, *Rpa70*, and *Ku80* genes expression decreased in the postnatal mouse testes from early to aged terms.

What is known already: The DSB levels occurring in the spermatogenic cells during spermatogenesis increase during biological aging in men. DSBs can be repaired by specific repair mechanisms including homologous recombination (HR) or non-homologous end joining (NHEJ) pathways. While RAD51 and RPA70 are the main components of HR process, KU80 and XRCC4 play important roles in NHEJ pathway. As is known, gH2AX is a commonly used biomarker for determining the DSBs.

Study design, size, duration: The potential reasons of DSB levels in the spermatogenic cells during aging is not fully addressed yet. In this study, we aimed to analyze the expression of the *Rad51*, *Rpa70*, *Ku80* and *Xrcc4* genes

at mRNA and protein levels in the postnatal mouse testes from early to aged terms.

Participants/materials, setting, methods: We comprised five groups based on the testicular histology, consisting of early (1- and 2-week-old), young (3- and 4-week-old), adult (5- and 6-week-old), late-adult (16-, 18- and 20-week-old), and aged (48-, 50- and 52-week-old). DSB repair genes expression at mRNA and protein levels were determined using qRT-PCR and immunohistochemistry techniques, respectively. The data were evaluated by using one-way ANOVA and Tukey post hoc test. $P<0.05$ was considered statistically significant.

Main results and the role of chance: The *Rad51*, *Rpa70, Ku80,* and *Xrcc4* mRNA levels significantly decreased in the aged group when compared to the young, adult and late-adult groups ($P<0.05$). gH2AX was intensively localized in the nucleus of primary spermatocytes of postnatal testes, and its levels either in the total or seminiferous tubules or germinal epithelial cells involving primary spermatocytes, round spermatids, elongating spermatids, elongated spermatids, and Sertoli Cells were higher in the aged group than the remained groups ($P<0.05$). The DSB repair proteins were detected in the spermatogenic cells, in which pachytene spermatocytes showed stronger intensity. The levels of RAD51 and RP70 proteins implicating in HR pathway were lower in the seminiferous tubules of the aged group when compared to the adult and late-adult groups ($P<0.05$). Moreover, the total and seminiferous tubules analysis revealed that KU80 levels decreased in the aged group in comparison to the remaining groups except for early group, as observed for the spermatogonia, primary spermatocytes and round spermatids of the aged group ($P<0.05$). Although there were no significant differences found in the total and seminiferous tubule analysis for XRCC4 protein, its levels decreased in the round spermatids and elongating spermatids of the aged group compared to the adult and late-adult groups.

Limitations, reasons for caution: The limitation of this study is that we did not isolate spermatogenic cell types from aged mice to compare adult ones.

Wider implications of the findings: The increase of DSB in the spermatogenic cells of aged mice may derive from reduced levels of RAD51, RPA70 and KU80 proteins playing roles in DSB repair. Further researches are required to determine the molecular mechanisms resulting in decrease of these protein levels.

Trial registration number: not applicable

O-212 MACS vs TESA for raised sperm DNA fragmentation index – a RCT

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Study question: In Individuals with raised Sperm DNA Fragmentation Index (SDF), will sperm selection by magnetic activated cell sorting (MACS) or surgical retrieval of testicular sperms (TESA) optimize the reproductive outcomes?

Summary answer: Couples with failed implantation raised SDF, TESA /MACS offer similar results. This RCT doesn't prove superiority or added benefit with any of the above interventions.

What is known already: It is evident that raised SDF negatively affects the reproductive outcomes. Management for raised SDF to optimize reproductive outcomes is still elusive.

Study design, size, duration: This was a Randomized Control Trial (RCT) with prior approval from institutional Ethical Committee and trial registration. Couples undergoing stimulation with raised SDF were randomized to MACS (n=75) and TESA (n=75) for sperm selection between April2019 & February2020.

Participants/materials, setting, methods: Couples with history of one failed IVF had SDF testing and SDF>30% were recruited. SDF test done with SCSA method and randomized using software. ICSI was the method of insemination. Extended embryo culture till blastocyst was done and freeze all policy was opted. Two Blastocysts that showed 100% survival were transferred in a Frozen Embryo transfer (FET) cycle. Embryonic and Reproductive outcomes were compared between both groups. Live birth and Miscarriage were the primary outcomes.

Main results and the role of chance: Reproductive Outcomes of MACS Vs TESA were:

Average Blastocyst conversion - 32% Vs 39% (RR 1.22, CI1.00 to 1.50)

Implantation rate (IR) - 50% Vs 35% (RR - 0.71, CI 0.51 to 0.98)

Miscarriage rate (MR) - 5.3% Vs 11% (RR1.6333, CI 0.5227 to 5.1039)

Multiple Pregnancy rate (MPR) - 8% Vs 4%

Live birth Rate (LBR) per Intention to treat (ITT) - 41.3% Vs 44% (RR 0.95, 95% CI 0.72 to 1.26)

LBR per ET cycle - 63% Vs 56% (RR 1.23, 95% CI 0.77 to 1.94)

Our preliminary results suggest that despite greater availability of blastocysts for transfer in the TESA group, no difference in ART outcomes was observed between the groups.

Though the IR was statistically low with TESA, our primary outcomes LBR and MR were comparable.

TESA or MACS seem to offer similar outcomes. Considering the invasiveness with TESA, MACS can be offered for better sperm selection for couples with raised sperm DFI & failed implantation.

Limitations, reasons for caution: Small sample size. TESA is a surgical intervention

Wider implications of the findings: Optimal intervention for management of SDF still needs further research.

Trial registration number: CTRI/2019/07/020140

SELECTED ORAL COMMUNICATIONS
SESSION 73: EMBRYO CULTURE AND DEVELOPMENT

01 July 2021 Stream 4 10:00 - 11:30

O-213 Slow day 5 development affects implantation potential of fresh transferred embryos but not birthweight once pregnancy occurs: A multi-center retrospective cohort study

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[6]Monash IVF Gold Coast, Embryology, Southport, Australia

Study question: Does slow development of fresh transferred day 5 embryos lead to decreased implantation potential and birthweight?

Summary answer: Slow day 5 development was associated with reduced implantation potential when transferred fresh but the subsequent birthweight of the resulting baby was not impacted.

What is known already: Slow development of *in vitro* cultured cleavage stage embryos is associated with reduced blastocyst development and implantation rates. There is no current consensus regarding whether to transfer fresh slow developing day 5 embryos or to extend culture for a subsequent day with potential for cryopreservation. It is therefore important to understand the true prognosis of fresh transferred day 5 embryos at less advanced developmental stages. This would provide evidence based guidelines for the decision making process in regard to embryo transfer.

Study design, size, duration: This is a retrospective multi-center cohort study, including 1213 consecutive patients undergoing autologous oocyte *in vitro* fertilization (IVF) treatment during 2016-2019,with fresh transfer of a single day 5 embryo (selection based on developmental stage and inner cell mass and trophectoderm morphology if blastocyst was at the ≥expanding stage). Cycle data were collected from 4 associated private clinics, with repeat cycles of same patients excluded to avoid clustering effect at statistical analysis.

Participants/materials, setting, methods: Live birth and birthweight were followed up in all 1213 fresh day 5 SETs. Multiple regression (logistic or linear) was performed to investigate association between slow day 5 development (defined as ≤ early blastocyst) and (a)live birth, (b) birthweight, and (c) gestation-adjusted birthweight (Z score) to account for gestational age, gender and

compared to embryos at ≥ expanded stage. Results were expressed as adjusted odds ratio (aOR) with 95% confidence interval (CI)or coefficients (β).
Main results and the role of chance: No implantation was achieved following single fresh transfer of day 5 embryos that failed to reach early blastocyst stage (n=76) and were transferred as ≤ morula stage. Live birth rate was significantly lower following single day 5 fresh transfer of an early blastocyst (n=237, 16%), in comparison to expanding (n=329, 27%, P=0.001), expanded(n=392, 41%, P=0.000), and hatching/hatched blastocysts (n=169, 44%, P=0.000). After adjusting for potential confounding factors including; maternal age, hours post insemination at day 5 assessment, number of oocytes collected, number of 2PN embryos, and number of embryos frozen; multiple logistic regression showed significantly reduced likelihood of live birth resulting from early blastocysts in reference to those at the expanding (aOR=0.584, 0.371-0.917, P=0.020), expanded (aOR=0.322, 0.208-0.501, P=0.000), or hatching/hatched stages (aOR=0.255, 0.147-0.443, P=0.000). However, multivariate linear regression indicated that early blastocysts resulting in a live birth (n=39) did not lead to altered birthweight (β=-9.091, P=0.904; β=-34.960, P=0.343; β=-26.074, P=0.414; respectively) or Z score (β=0.045, P=0.706; β=-0.051, P=0.426; β=-0.028, P=0.506; respectively) in reference to the expanding (n=90), expanded (n=160), or hatching/hatched stages (n=75).
Limitations, reasons for caution: The retrospective nature of this study does not allow controlling of unknown confounders. The 4 participating clinics are associated within the same network with shared protocols, therefore, results may not be generalized to other clinics with different settings.
Wider implications of the findings: The findings suggest no clinical value of fresh day 5 transfer of embryos ≤morula stage. Although early blastocysts implant at reduced rate, assuring birthweight outcomes suggest clinical value. Future studies intend to investigate slow growing day 5 fresh transfers versus embryos that were slow growing but transferred after day 6.
Trial registration number: NA

O-214 Undisturbed embryo culture under High Humidity atmosphere in a time-lapse system increases pregnancy rates

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Study question: Does culture in high relative humidity conditions (HC) improve pregnancy rates when using a time-lapse system (TLS) and single-step (SS) culture medium?
Summary answer: Using an integrated-TLS and SS medium, culture under HC increases the likelihood of embryos to achieve a pregnancy with respect to those cultured in DC.
What is known already: Many variables affect embryo development, and need to be precisely tuned in every IVF laboratory, especially inside the incubators. TLS provide stability during embryo culture, which is a well-known key factor for a proper embryo development. The humidity content of culture atmosphere is especially relevant in order to avoid oscillations in culture media osmolality. It has been previously reported that culture under HC has a significant effect on embryo quality and morphokinetics. However, studies assessing the effect of HC in clinical outcome are rare and inconclusive, mostly due to the variability in the incubator device used and insufficient sample size.
Study design, size, duration: The present is a retrospective study performed over 1624 ICSI treatments from 3 fertility clinics from December 2017 to October 2020. Zygote cohorts were randomly assigned to dry (N=794) or humid conditions (N=830). It includes autologous treatments with (N=555) and without (N=368) pre-implantation genetic testing (PGT) and egg donation treatments (N=701). Following selection by combining morphological and morphokinetic criteria, 1611 mostly single embryo transfers (92%) were performed, 779 from DC and 832 from HC.
Participants/materials, setting, methods: Stimulation, oocyte pickup and fertilization were performed according to the standard procedures of the clinic. We used a GERI incubator (Genea Biomedx), with 6 separated chambers for individual patients, 3 of them configured to work in DC, and 3 in HC. Embryos were cultured in specific 16-well GERI trays with single-step Gems® culture medium (Genea Biomedx). The effect of HC in pregnancy rate was assessed by multivariate logistic regression and Pearson Chi Square Test.
Main results and the role of chance: Types of treatment and patient demographics were homogeneously distributed in the two study groups. Mean patient age was 39.88±4.47 years, BMI: 23.54±4.21 Kg/m2 and number of correctly fertilized oocytes: 7.86±3.87. A logistic regression was performed, including other possible affecting factors: ovum age and origin, transfer day, fresh or frozen-warmed embryo transfer, number of transferred embryos and the use of PGT. Said analysis revealed that embryos cultured in HC are more likely to achieve a pregnancy than those cultured in DC (OR=1.30, 95% CI (1.05-1.59), p=0.014). Pregnancy rate was significantly higher in HC (66.7%) than in DC (60.9%) in the total embryo transfers (p=0.017). Pregnancy rate was also higher in HC in fresh embryo transfers (68.6% in HC vs 63.2% in DC; p=0.133) and frozen-thawed transfers (65.2% in HC vs 59.1% in DC; p=0.062), although differences were not statistically significant due to the reduced sample size. Stratifying the results, the significant difference remained in transfers belonging to autologous cycles (68.4% HC vs 56.5% in DC; p=0.030) and in treatments in which PGT was performed (67.1% HC vs 56.0% in DC; p=0.023), but the difference in egg donation procedures was not statistically significant (66.4% in HC vs 64.7% in DC, p=0.577).
Limitations, reasons for caution: This is a retrospective analysis performed over the clinics' treatments, so it might be compromised by some bias, although multivariable analysis may overcome them. For further assessing the effect of HC in clinical results a prospective controlled study, with a larger sample size could be performed, also comparing life-birth rates.
Wider implications of the findings: These results, alongside our previous findings (Valera *et al*. 2020, Albert *et al*. 2020), support that HC contributes to optimize embryo development and clinical results in undisturbed culture in TLS with single-step medium. To our knowledge, this is the largest study on the matter and the first performing multivariable analysis.
Trial registration number: Not applicable

O-215 How common is add-on use and how do patients decide whether to use them? A national survey of IVF patients

S. Lensen[1], K. Hammarberg[2], A. Polyakov[3], J. Wilkinson[4], S. Whyte[5], M. Peate[1], M. Hickey[1]

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Study question: How common is IVF add-on use in Australia, and what drives the use?
Summary answer: Most women (82%) had used one or more IVF add-ons and more than half (54%) first learned about the add-ons from their fertility specialist.
What is known already: IVF add-ons are procedures, techniques or medicines which may be considered nonessential to IVF, usually used in attempts to improve the probability of conception and live birth. Despite widespread concern about unproven IVF add-ons, information about the prevalence of their use is limited because these data are not available in national registries or datasets.
Study design, size, duration: Women who had undergone IVF in Australia since 2017 were recruited via social media. Women were excluded if they were gestational surrogates, had used a surrogate, or underwent IVF for oocyte donation or elective oocyte cryopreservation only. Eligible women completed an online survey which was open from 21st June to 14th July 2020.
Participants/materials, setting, methods: Survey questions included demographics, IVF and medical history, and questions specifically about IVF add-ons such as: the type of add-ons used, information sources consulted, and where participants first heard about add-ons. Women also responded to questions about the importance of scientific evidence regarding safety and

effectiveness, factors considered in decision-making around add-on use and the presence of any decision regret.

Main results and the role of chance: A total of 1,590 responses were analyzed after excluding 287 ineligible responses. Participants were generally representative of women who undergo IVF in Australia in terms of age, indication for IVF, and use of ICSI for fertilisation. Most women had used at least one add-on (82%), and these were usually associated with an additional fee (72%). It was most common to first learn about IVF add-ons from the fertility specialist (54%), and most women reported that they and their specialist contributed equally to the decision to use add-ons.

Women viewed scientific evidence for safety and effectiveness as very important: on a scale from 0-100, an importance score over 90 was selected by more than half of the participants. Additionally, many (49%) assumed that add-ons were risk-free. Most women experienced regret at the decision to use IVF add-ons (66%), and this regret was greatest among women who experienced IVF failure when using add-ons (83%) and those who believed that the specialist drove the decision to use the add-ons (75%).

Limitations, reasons for caution: This was a retrospective survey of IVF patients, therefore it may suffer from bias due to patient recall. It does not consider the perspective of the IVF clinic or fertility specialist. Certain questions may be more prone to biased responses, such as those regarding who contributed to decision making.

Wider implications of the findings: The high prevalence of add-on use is likely generalizable to other settings where IVF treatment is largely private. Although women viewed scientific evidence as very important, most had used unproven IVF add-ons. This might suggest that women were not aware of the lack of robust evidence to support their use.

Trial registration number: Not applicable

O-216 Culture medium used in IVF-treatment impacts post-implantation embryonic growth and developmental trajectories in a sex-specific manner

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[4]Erasmus MC- University Medical Centre, Obstetrics and Gynaecology - Division of Reproductive Endocrinology and Infertility, Rotterdam, The Netherlands

Study question: What is the (sex-specific) impact of two different culture media used in *in vitro* fertilization (IVF) treatment on post-implantation growth and development?

Summary answer: Embryos, especially males, cultured in SAGE 1-Step grow and morphologically develop faster in the first trimester, when compared to those cultured in Vitrolife G-1 PLUS.

What is known already: Increasing success rates after IVF can be attributed to several advancements, such as improved culture conditions. Culture media are of special interest, as they supply the embryo with essential nutrients and have previously been shown to impact birthweight. Moreover, IVF pregnancies are associated with an increased male:female ratio. However, it is unknown if culture media also have an impact prenatally. Therefore, our aim is to study the (sex-specific) impact of two different culture media (SAGE 1-Step and Vitrolife G-1 PLUS) used in IVF treatment on first-trimester embryonic growth and development, and fetal outcomes.

Study design, size, duration: Women with a viable singleton pregnancy were included before 10 weeks of gestation in the Rotterdam Periconception Cohort, an ongoing prospective tertiary hospital-based study, conducted since November 2009.

Participants/materials, setting, methods: A total of 879 pregnancies were included; 153 after culture in Vitrolife G-1 PLUS, 251 after culture in SAGE 1-Step and 475 naturally conceived. First-trimester growth and development, defined by serial crown-rump length (CRL), embryonic volume (EV) and Carnegie stages measurements were performed using state-of-the-art imaging techniques. Secondary outcomes included second trimester estimated fetal weight (EFW) and birth outcomes, and were retrieved from medical records.

Main results and the role of chance: Linear mixed model analyses, adjusted for gestational age and maternal characteristics, showed that embryos cultured in SAGE 1-Step grow faster than those cultured in Vitrolife G-1 PLUS (β_{EV} 0.030 $\sqrt[3]{cm^3}$ (95%CI 0.008-0.052), p=0.007). CRL and Carnegie stages were not statistically different between culture media. After stratification for fetal sex, similar results were observed for male embryos (β_{EV} 0.048 $\sqrt[3]{cm^3}$ (95%CI 0.015-0.081), p=0.005), but not for female embryos. EFW and birth outcomes were comparable between culture media in the total population and after stratification for fetal sex. Embryos cultured in SAGE 1-Step also grow faster than those conceived naturally (β_{EV} 0.033 $\sqrt[3]{cm^3}$ (95%CI 0.006-0.060), p=0.018). This association was also most pronounced in male embryos (β_{EV} 0.066 $\sqrt[3]{cm^3}$ (95%CI 0.024-0.108), p=0.002).

Limitations, reasons for caution: Although this study has a prospective design, its observational character does not exclude residual confounding. Furthermore, the external validity of this explorative study is limited, since participants were recruited from a tertiary university hospital.

Wider implications of the findings: Culture in SAGE 1-Step culture medium is associated with faster first-trimester growth and development, especially in male embryos. This may be the result of altered susceptibility to preimplantation environmental stressors. Further research should focus on the (sex-specific) impact of culture media on postnatal development and the susceptibility to non-communicable diseases.

Trial registration number: N/A

O-217 Bitter Taste Receptors expression in human follicular cells: new perspectives in female fertility

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Study question: Bitter TasteReceptors (TAS2Rs)role in female reproductive system cells: potential implications in mechanisms underlying oocyte maturation and sperm-oocytes interaction.

Summary answer: TAS2Rs and genes involved in their transduction cascade are differentially expressed in granulosa (GCs)and cumulus cells(CCs).

What is known already: TASRs expression can be found also in extraoral location wherein their function appears less obvious. TASRs are reported to be involved in signal transduction cascade induced by chemotactic activation in spermatozoa and the expression of TAS2Rs in ejaculated human sperm has been demonstrated. The presence of these receptors in male reproductive system and in sperm gives cues to investigate their possible role in sperm-oocyte interaction.Functional implications have been collected indicating that taste receptors are also important to increase the number of highly fertilization-competent sperm cells within the female genital tract hypothesizing a role in the field of female reproduction.

Study design, size, duration: We enrolled for this study 30patients undergoing IVF cycles because of couple infertilityfrom June 2019 to October 2020at the UOSA of Assisted Reproductive techniques, Siena University Hospital(Italy).

Participants/materials, setting, methods: Female patients referring to UOSA of Assisted Reproductive techniques(median age 35 years) underwent a personalised controlled ovarian hyperstimulation protocol. After oocyte pickup, GCs were isolated from the follicular fluid through differential gradient. CCs were collected after oocytes denuding. TAS2Rs and genes involved in the transduction cascade elicited expression/localization in both GCs and CCs were confirmed by Droplet Digital PCR, western blot andimmunofluorescence.

Main results and the role of chance: For the first time, the expression and cellular localization of the TAS2Rs (TAS2R3, TAS2R4, TAS2R14, TAS2R19 and TAS2R43), their G-coupled proteins (GNAT1 oro-transducinandGNAT3 or α-gustducin) and enzymes involved in the transduction signal (PDE4A, TRPM5 and PLCB2)were demonstrated in the female reproductive system.Overall expression of TAS2Rs emerged higher in GCs than in CCs, confirming the specific molecular fingerprinting during differentiation of ovarian somatic cells. TAS2R14 is the most expressed gene in both GCs and CCs, this could account for its potential involvement in follicular cells physiology and/or for a key role of this receptor in fertilization, as supported by data showing TAS2R14 to be

correlated with sperm progressive motility. We demonstrated a positive correlation in GCs between the expression of the TAS2Rs and GNAT3; interestingly, when each subset of TAS2Rs genes was correlated with the signaling gene, TAS2R14 emerged as the one with the higher correlation with GNAT3. Immunofluorescence showed different localization of TAS2Rs and their G-coupled proteins between GCs and CCs. Interestingly some of them presented some fluorescent granules, suggesting a possible involvement of proteins in membrane trafficking. Finally, results of G-coupled proteins western blot, revealed the higher expression of -gustducin then -transducin, confirming the gene expression.
Limitations, reasons for caution: All findings have to be validated in a larger cohort. Moreover, our data pave the way to the understanding of biological functions exerted by these receptors in the female reproductive tract.
Wider implications of the findings: Further studies might contribute to better understanding the physiologic role of taste receptorsfemale reproductive system. This should becrucial to clarify the role of these receptors in maturation or competence acquiring of oocytes, or also in sperm-oocytes attraction and recognition, crucial point in fertilization process
Trial registration number: Not applicable

O-218 The effect of mTOR activation on human primordial follicle activation during in-situ culture

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Study question: What is the effect of mTOR pathway activation on human primordial follicles in-situ activation and subsequent development following tissue cryopreservation?
Summary answer: Temporary treatment of cryopreserved human ovarian tissue with mTOR activators cause the initiation of primordial follicle development and influence steroidogenesis.
What is known already: In-vitro activation of primordial follicles to produce mature oocytes provides an alternative technique for fertility preservation. The employment of different stimulators of PI3K pathway has been successfully used to activate resting follicles during culture or prior to grafting in patients with premature ovarian insufficiency. The addition of phosphatidic acid (PA) and propranolol (PP), as mTOR stimulators, in the culture medium has promoted primordial follicle activation morphologically in mouse and human ovaries. Molecular and functional evaluations of primordial follicle activation after treatment with the mentioned stimulators has not been conducted.
Study design, size, duration: Ovarian tissues which were donated from 6 transsexual patients (23-35 years old), were dissected and cryopreserved with slow-freezing technique. After thawing, they were cut into 1×1×1 mm fragments and incubated with different stimulators in three groups: 1) Control (without stimulators), 2) PA (200μM), and 3) PA+PP (200μM & 50μM respectively). In groups two and three, ovarian fragments were cultured for 24 hours in presence of stimulators and then cultured for additional 6 days without stimulators.
Participants/materials, setting, methods: The cultured ovarian fragments were directly processed for Hematoxylin and Eosin staining and Western blot analysis. The proportion of morphologically normal and degenerated primordial and growing follicles and 17β-estradiol (E2) level in the culture medium were compared after 1 and 7 days of culture to assess follicular development and function. Western blot analysis for phosphorylated and non-phosphorylated status of FOXO3a and RSP6 proteins expression were compared after 24 hours of incubation.
Main results and the role of chance: The proportion of primordial and growing follicles were not significantly different in the experimental groups after 24 hours of incubation with either of the stimulators. Western blot analyses indicated a significant reduction of FOXO3a in the PA+PP group compared to the control group. The phosphorylation level of RPS6 protein did not significantly change in either of the groups. The proportion of transitional follicles were significantly higher in the PA group compared to other groups after 7 days of culture. The E2 secretion level was significantly higher at the last day of culture compared to day 1 for all groups. At the end of the culture period, E2 levels was significantly higher in both PA and PA+PP groups compared to the control group.
Limitations, reasons for caution: Due to ovarian fragmentation before culture, the HIPPO pathway downstream molecules should have also been evaluated by western blot, which was not contributed in this study.
Wider implications of the findings: The results demonstrate the beneficial effect of mTOR signaling to accelerate early primordial follicle recruitment in cryopreserved-thawed human ovarian fragments.
Trial registration number: not applicable

POSTER DISCUSSION
SESSION 74: ETHICS AND LAW POSTER DISCUSSIONS
1 July 2021 Stream 1 11:45 - 12:30

P-351 Ethical challenges posed by an increase of surplus frozen embryos in Argentinean fertility centers

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Study question: Can an increase in the quantity of frozen embryos lead to more difficulties in embryo disposition decisions (EDD)?
Summary answer: EDD posed clinical and ethical challenges and might be influenced by having more available embryos, due to changes in the laboratory procedures.
What is known already: Previous research suggests that many people find EDD difficult and emotionally distressing. Patients face ambivalence during the decision-making process which could lead to embryo abandonment. Regulation of embryo dispositions varies among countries, but in the Latin American context, the regulatory gap generates insecurities in healthcare professionals. Cultural values towards the embryo can be associated with discomfort, guilt, or psychological burden. Studies suggest that patients often feel that they are unable to make a satisfactory decision when presented with the current embryo disposition options. Thus, other 'solutions', such as the request for nonreproductive transfer, appear and raises ethical questions and concerns.
Study design, size, duration: This is an observational study which follows a thematic literature review, that identifies the main reasons for difficulties with embryo disposition decisions, in different countries. It focuses on the regulatory background of the principal ART providers worldwide to discuss the best course of action for Argentina, that faces the problem of EDD at a regulatory level. To inform the discussion, a comparative survey from an Argentinean context, was carried out.
Participants/materials, setting, methods: Most fertility clinics in Argentina are private entities, as there are very few public providers. Access to ART treatments has been regulated since 2013, but the law fails to define a number of important issues, including EDD and national registries. An online survey was sent to all reproductive facilities to collect data on storage content and the results were complemented with data from the Latin American Register (RedLara) and the Argentine Registry of Assisted Fertilization.
Main results and the role of chance: The survey results showed that in 2017, there were approximately 54.432 frozen embryos stored in 46 Argentinean fertility centers and the total amount in 2020 reached 91.724 stored in 54 centers. Despite the number of treatment cycles (IVF + OD) being constant between 2017 and 2020 (with a slight increase of 8%), the number of frozen embryos has increased exponentially (by 68.5%). This is a consequence of the improvements in cryopreservation techniques (vitrification) and the development of more efficient ovarian stimulation protocols, that have facilitated a rise in elective single embryo transfer. These advances, coupled with an inefficient regulatory

framework, generate uncertainties in physicians who might already be conflicted and therefore provide little or inadequate guidance for patients facing EDD. Three strategies could be implemented to facilitate EDD under this particular setting. First, counseling sessions at different treatment stages should be encouraged and are conducted by trained mental health professionals, who are aware of their patient's changing attitudes towards surplus embryos. Second, both aneuploid embryos and embryos which were cryopreserved more than 10 years ago could form part of a national bank for research purposes, using classified storage content. Third, promote effective regulation that includes EDD and explicit storage limits.

Limitations, reasons for caution: The influence of the Catholic Church on policy makers regarding embryo dispositions is the main drawback. There is a need to foster a regulatory framework that considers the changes in IVF procedures and practices. On a practical level, psychosocial care is missing as part of healthcare teams' practices.

Wider implications of the findings: The survey results revealed that IVF centers in Argentina will face an increase in euploid, aneuploid and untested frozen embryos, due to the changes registered in laboratory procedures. This tendency shows the need to discuss EDD with patients from the beginning of fertility treatment, through to its conclusion.

Trial registration number: not applicable

P-352 Beyond individualisation: towards a more contextualised understanding of women's social egg freezing experiences

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Study question: What are the moral perceptions and views of women considering social egg freezing?

Summary answer: Participants did not perceive egg freezing as a morally problematic solution to societal problems but addressed concerns about relationship formation and wanted more social efforts.

What is known already: Central to the social egg freezing debate is the individualisation argument which underlines the idea that it is morally problematic to use individual medical-technological solutions, such as egg freezing, to solve the societal challenges women face, for instance in the current labour market. It has been said that, instead of quick medical-technical solutions that target individual women's bodies, we should focus on substantive changes that target the androcentric work culture. This theme relates to feminist concerns about unnecessary medicalisation geared towards women. Furthermore, there is a call for more empirical studies to back up this central normative claim.

Study design, size, duration: Seventeen participants were recruited by psychologists working in two Belgian centres for reproductive medicine which offer egg freezing for social reasons. In addition, four participants were recruited through via social networks. Interviews took place between February 2019 and November 2020 at a location of the participants' preference or through online video connections.

Participants/materials, setting, methods: At the beginning of the interview, open questions were asked to invite the participants to speak about social egg freezing in their own words. In the second part of the interview, we used four cards with controversial statements based on a study of the bioethics literature, to encourage the participants to reflect about ethical concerns. In this part, we engaged in Socratic dialogue. For the analysis, thematic analysis was used combined with interdisciplinary collaborative auditing.

Main results and the role of chance: This is the first study providing empirical evidence about (potential) egg freezers' moral reasoning about individualisation arguments. Most participants in our study could make sense of the individualisation argument but emphasised another societal challenge rather than the current labour market. They highlighted 'the lack of a partner relationship' as driving their motivation for this procedure. The shortage of eligible partners has been well defined in social science scholarship about social egg freezing but this element has rarely been articulated in the premises of individualisation arguments. This topic of relationships is challenging to analyse from a normative perspective because it was experienced as much more personal and intimate by the women in our study than for instance measures to realise more fair labour conditions, such as improved access to childcare. Some participants believed egg freezing resulted from individual problems and found the individualisation argument not applicable to their own situation. Furthermore, no participant found the individualisation argument legitimate to depict social freezing as morally problematic. Nonetheless, the participants showed a sense of sympathy with women who lack access to egg freezing and were in favour of societal solutions in several public domains.

Limitations, reasons for caution: Given that we report on a small-scale qualitative study of possible social egg freezers at two Belgian fertility clinics, and that our study foregrounds the voices of mostly white higher educated women who were able to afford this technology, our results cannot be generalised to all social egg freezers.

Wider implications of the findings: Our findings can contribute to a better understanding of previously identified normative arguments (e.g., individualisation and unnecessary medicalisation). There is a definite need to further analyse the complex interplay between respecting autonomous choices and evaluating contextual factors in this debate and other practices where similar individualisation arguments are used.

Trial registration number: Not applicable

P-353 When Parents and Minor Children Disagree about Fertility Preservation: A Scoping Review and Ethical Analysis

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Study question: Periodically, parents and children disagree about whether to pursue fertility preservation (FP). How should medical teams navigate these ethically complex situations?

Summary answer: Several considerations must be weighed, including the minor's age, the burden of the proposed procedure, and whether the minor or parent seeks to decline FP.

What is known already: As reproductive technology advances, FP prior to gonadotoxic therapy has become the standard of care. Periodically, parents and children disagree about whether to pursue FP. To date, there is no clear guidance on how to navigate these difficult situations. Prior studies have demonstrated that adolescents undergoing gonadotoxic therapy want their views regarding FP to be taken into account, and also that most children and adolescents are comfortable with parental involvement in decision-making. However, transgender adolescents pursue FP at lower rates than adolescents with cancer, and more research is required to elucidate the unique needs and barriers of transgender youth.

Study design, size, duration: This study involves a scoping review and ethical analysis about parent-child disagreement regarding FP in minors. The review analyzes papers that either demonstrate that parent-child disagreement occurs, describe the preferences of parents or children regarding decision-making around FP, or provide recommendations that can be used to resolve parent-child conflicts. The ethical analysis weighs relevant rights and interests, including the child's best interest, the right to an open future, the child's autonomy, and parental autonomy.

Participants/materials, setting, methods: A search string was developed to identify all relevant published manuscripts on the topic of FP in minors, including studies on decision-making, family relations and ethical challenges. The search was run through several databases, abstracts were screened using Covidence, and data were extracted from full texts. Data abstracted from the review and existing literature on general medical decision-making for minors were used to construct an ethical framework for parent-child disagreements regarding FP in minors.

Main results and the role of chance: Published work directly on the topic of parent-child disputes regarding FP is limited, however a number of studies tangentially discuss parent-child disagreements and provide insight into the desires of parents and children regarding decision-making around FP. Studies suggest that adolescents desire to have their views taken into account, and a minority of adolescents believe their wishes alone should be followed. The age of the minor is a crucial factor, and some propose that as adolescents approach

adulthood, their autonomy should increase. At the same time, in practice, legal and financial constraints often render parents the ultimate decision-makers. Our ethical analysis weighs competing considerations, including the child's best interest, the right to an open future, the child's autonomy, and parental autonomy. It concludes that who prevails should depend on contextual factors, including the minor's age, the burden of the proposed procedure, and whether the minor or parent seeks to decline FP. There may also be special considerations for transgender adolescents, some of whom might have deeply personal reasons for pursuing or forgoing FP that are not well-understood by cisgender parents.
Limitations, reasons for caution: The scoping review captured a variety of results, including survey and interview studies, society guidelines, and ethical analyses. As such, we were unable to define a uniform quality metric. However, we aimed to be more rather than less inclusive because of the limited results directly pertaining to parent-child disagreements.
Wider implications of the findings: This study provides a robust review of decision-making for FP in minors and offers an ethical framework for weighing countervailing considerations when parents and children disagree about whether to pursue FP. The conclusions can be used to inform guidance for clinicians presented with this challenging ethical dilemma.
Trial registration number: N/A

POSTER DISCUSSION

SESSION 75: REPRODUCTIVE SURGERY POSTER DISCUSSIONS

1 July 2021 Stream 2 11:45 - 12:45

P-745 The efficacy of Buscopan® in reducing pain during ultrasound-guided manual vacuum aspiration (USG-MVA): A double-blind randomised placebo-controlled trial

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Study question: Does Buscopan® reduce abdominal pain experienced by women undergoing ultrasound-guided manual vacuum aspiration (USG-MVA)?
Summary answer: The addition of 20mg Buscopan® intravenous injection was not associated with a statistical reduction in pain score but leads to a higher patient satisfaction score.
What is known already: Ultrasound-guided Manual Vacuum aspiration is a feasible and effective out-patient treatment option for treating early pregnancy loss. However, it is associated with a moderate amount of pain due to uterine contraction.
Study design, size, duration: This randomised, double-blinded, placebo-controlled trial was conducted in a university-affiliated tertiary hospital. The study assessed whether 1 ml of 20mg Buscopan® intravenous injection 5 minutes before the USG-MVA will reduce the abdominal pain experienced by the women immediately and 2 hours after the procedure. Participants were randomised between June 2018 to January 2020 using a computer-generated number series in a 1:1 ratio.
Participants/materials, setting, methods: Women aged 18 years or older with first-trimester miscarriage undergoing the USG-MVA procedure were eligible. In total, 122 participants out of 128 eligible were included. Of whom, 111 underwent the USG-MVA procedure, 60 randomised to the Buscopan® group, and 62 to the placebo group.
Main results and the role of chance: The median abdominal pain scores in the Buscopan® group were 16.0% and 21.2% lower than the placebo group immediately post-procedure and 2 hours after the procedure in the Buscopan® group. Repeated measures ANOVA indicated that the both vaginal and abdominal pain scores improved significantly with the time (Vaginal $F(1,108)= 180.1, p<0.0001$;
Abdominal: $F(1,108)=83.41, p<0.001$) but not with group. No difference was noted in the complications and side effects profile. The physiological stress measured by Log10 sAA levels reduced significantly with time ($F(2.8,286.1)= 6.3$, $p<0.001$) but not with group ($F=0.1$, $p=0.96$). Women randomised to Buscopan® had a significantly higher ($p=0.032$) mean VAS satisfaction scores compared to those receiving placebo (79.0±17.3 vs 73.4±24.1).
Limitations, reasons for caution: This study was a single-centre study, thus one should be cautious in the overall generalisability of the results.
Wider implications of the findings: Few studies have evaluated the use of anti-spasmodic agents to minimise uterine contraction pain in women undergoing outpatient uterine evacuation. We consider Buscopan® a useful adjunct in the pain control of USG-MVA to specifically reduce uterine cramps. Further larger studies are required to evaluate its efficacy
Trial registration number: ChiCTR1800014590

P-746 Obstetric outcomes of singleton birth after hysteroscopic division of septate uterus

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Study question: The aim of this study is to determine the obstetric outcomes in patients with a singleton birth after hysteroscopic division of septate uterus.
Summary answer: The data suggest excellent obstetric outcomes for singleton gestation after hysteroscopic division of a septate uterus reaching either the internal or the external cervical os.
What is known already: Septate uterus is a rare Müllerian anomaly with major impact on reproductive outcomes, particularly with a septum over 10mm. Controversy still exists over the need for surgical correction of the septum due to conflicting data on outcomes, particularly in women with histories of good obstetric outcomes and incidental septum findings. Placental location in relation to the septum may account for such conflicting reports. Most data on reproductive outcomes after hysteroscopic surgical correction combine both septate and subseptate uteri. There is limited published data on obstetric outcomes after hysteroscopic surgical correction of septate uteri, especially septate uteri reaching the external os.
Study design, size, duration: This retrospective cohort study included 107 patients with infertility and/or recurrent pregnancy loss (RPL) who received treatment between 2002 -2019. The study group included 24 patients with a singleton birth after hysteroscopic correction of septate uterus (Class Va; ASRM classification) that was diagnosed on trans-vaginal 3D ultrasound. The control group included 83 patients with a singleton birth who had normal endometrial cavity on hysteroscopy during the same period of time, before starting treatment.
Participants/materials, setting, methods: This study was conducted at an infertility clinic affiliated with a teaching hospital. In the study group the septum reached the internal or the external cervical os in 14 and 10 patients respectively. After hysteroscopic correction, all patients were offered various infertility treatments depending on the underlying etiology. The inclusion criterion in this study was to have a singleton birth after hysteroscopy. Demographic and clinical data and obstetric outcomes were compared between the two groups.
Main results and the role of chance: There was no significant difference in mean age, infertility duration, infertility type and incidence of male infertility or ovulatory disorders between the two groups. There was a significantly higher BMI (0.048), and a higher incidence of history of miscarriage ($P=0.002$) and history of RPL ($P=0.017$) in the study group. There was significant lower incidence of tubal factors infertility ($P=0.005$) and endometriosis ($P=0.03$) in the study group, therefore there was higher incidence of spontaneous conception (70.8% vs 19.3%; $P=0.000$) and lower incidence of conception with IVF-ET (20.8% vs 66.3%; $P=0.000$) in the study group compared to the control group respectively. There was significantly higher incidence of prophylactic cervical cerclage (17.4% vs 0%; $P=0.000$), and delivery by CS (69.6% vs 41.2%; $P=0.019$) and lower incidence of vaginal delivery (30.4% vs 58.8%; $P=0.019$), in the study group compared to the control group. There was no significant difference in

gestational age in weeks (38.3+1.8 vs 38.6+2.0), newborn birth weight in grams (3173.9+630.0 vs 3202.1+555.6), incidence of premature birth (12.5% vs 12.2%), or other obstetric complications (25% vs 17.6%) between the study and the control groups respectively. For premature births, mean gestational age was 34.3+0.47 and 34.6+1.2 weeks in the study and control groups respectively.

Limitations, reasons for caution: A retrospective study has its own inherent bias. Furthermore, the small sample size is explained by the fact that a septate uterus is a rare anomaly leading to difficulties finding cases and organizing a prospective study to achieve a larger sample size. A multicenter prospective study is needed.

Wider implications of the findings: Regardless of whether the septum reached the internal or external os, there were excellent obstetric outcomes in singleton gestations after hysteroscopic correction of septate uteri. There was no increased risk with septate uteri involving the cervix. Hysteroscopic surgical correction should be the treatment of choice for patients with septate uteri.

Trial registration number: Not Applicable

P-748 Diode laser hysteroscopic metroplasty for dysmorphic uterus: a pilot study

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Study question: Whether diode laser hysteroscopic metroplasty for dysmorphic uterus is a safe and efficacious procedure and its effect on reproductive outcomes.

Summary answer: Diode laser hysteroscopic metroplasty is a safe and effective procedure for infertile women with dysmorphic uterus with comparable results to those reported in the literature.

What is known already: A T-shaped uterine anomaly is categorized by the ESHRE/ESGE consensus as dysmorphic uterus class U1a, characterized by an abnormal hypoplastic uterine cavity. A Y-shaped uterus is a dysmorphic uterus with a fundal subseptum. Dysmorphic uteri are associated with infertility, recurrent implantation failure (RIF), recurrent pregnancy loss (RPL), and adverse pregnancy outcomes. According to several studies, it seems that hysteroscopic metroplasty may improve the chances of conception and live birth. Previous studies described the procedure using bipolar systems, monopolar needle or scissors. The purpose is to achieve a uterine cavity of normal shape and volume by cutting the thickened lateral walls.

Study design, size, duration: This was a retrospective pilot study with a prospective follow-up. We retrospectively evaluated all cases operated between February 2018 to February 2020, at Hillel Yaffe Medical Center, Hadera, Israel. Reproductive outcomes for women who underwent the procedure were followed until September 2020. Pregnancies that were ongoing on September 2020 were followed until January 31st 2021.

Participants/materials, setting, methods: Nulliparous women with a diagnosis of infertility or RPL, who were diagnosed with dysmorphic uterus by three-dimensional ultrasound (3D-US) and underwent diode laser hysteroscopic metroplasty were included. All the metroplasties were done in one tertiary center by the same specialist. Reproductive outcomes were evaluated retrospectively and prospectively for a total follow-up time of 32 months. Reproductive performances before and after metroplasty were compared where possible.

Main results and the role of chance: Twenty-five women underwent diode laser hysteroscopic metroplasty for dysmorphic uterus in our institute. No perforations, excessive bleeding, or other complications were encountered during the procedures. Follow-up hysteroscopy and 3D-US were satisfactory in all cases 2 months after the metroplasty. A total of 15 nulliparous women returned to fertility treatments afterwards, among whom 9 conceived (60% pregnancy rate). Their infertility period before the procedure was 56.6 ± 36.1 months. The duration between the metroplasty to pregnancy was 5.2 ± 3.5 months. The rate of deliveries and ongoing pregnancies (pregnancies beyond 20 weeks of gestation) was 78% (7/9), with five successful liveborn deliveries and two ongoing pregnancies. All deliveries were between 36-37 weeks. The 10 women who were not treated by our infertility unit were contacted, among whom 6 discontinued their attempt to conceive. The other 4 conceived; three of them spontaneously. Among those 4 women,the rate of deliveries and ongoing pregnancies was 75%, with one term delivery and two ongoing pregnancies.

Limitations, reasons for caution: First, we included both T-shaped and Y-shaped uteri as both represent close versions of dysmorphic uteri, but in fact they differ. The subseptum might interfere with reproduction in a different mechanism. Second, the small and heterogeneous sample as well as the short duration of follow-up limit the conclusions.

Wider implications of the findings: We present the first application of diode laser in hysteroscopic metroplasty for dysmorphic uteri. This technique seems promising and our results are comparable with other series using different cutting devices. Only larger controlled trials with a longer follow-up can confirm the safety, efficacy, and impact on reproductive outcomes.

Trial registration number: Not Applicable

P-750 Clinical efficacy of virtual reality for acute pain and anxiety management during outpatient hysteroscopy and endometrial biopsy in subfertile patients

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Study question: Does the use of virtual reality (VR) headsets in diagnostic office hysteroscopy (HSC) with endometrial biopsy (EB) reduce anxiety and pain scores in the patient?

Summary answer: Virtual reality during office HSC do not seem to improve relaxation, anxiety, or pain scores. Physicians have a good perception of patients' pain.

What is known already: Women undergoing outpatient HSC experience high levels of preoperative anxiety, which increase pain and discomfort during the procedure. The experience of pain is a complex phenomenon, which simultaneously occurs on cognitive, emotional, and behavioural levels, and is influenced by many factors. A Cochrane review failed to show a significant difference between different types of pain relief (analgesics, local anaesthetic and verbal support techniques …). VR is a multisensory immersion providing an interactive high level distraction, occupying a large portion of humans' finite attentional resources (vision and audio), and leaving less cognitive capacity available to process pain.

Study design, size, duration: The sample size for this prospective randomized controlled trial was calculated at 196 patients (98 per group), considering a power of at least 80% to detect superiority of adding a VR headset versus standard care, standard deviation (SD=2.0), using a two-sided, t-test, at significance level alpha of 0.05.

The preliminary results after 1 month include a sample of 48 patients: 25 in the VR group and 23 controls.

Participants/materials, setting, methods: All 48 patients suffer subfertility and underwent HSC with EB at our tertiary-care fertility center. We used Oncomfort®, a commercially available VR autohypnosis relaxation program designed for perioperative settings. The headmounted smartphone display with headphones provides image sound distraction with suggestive hypnosis techniques incorporated. Before and immediately after the exam, both patients and surgeons fill out a questionnaire using the 10.0cm visual analog scale (VAS).

Main results and the role of chance: The mean duration of HSC was 3min43sec in the VR group, (range 2-6min), compared to 4min50 in the control group (range 1-12minutes), which was not significantly different (p=0.09). Subjective variables of stress, anxiety and pain were evaluated at four different time points, i.e. before, during, immediately after HSC and one week later.

According to VAS, stress levels did not differ significantly (p>0.05) between the VR group and the control group, or within time: 5.08 to 5.36 to 3.08 vs 4.48 to 4.83 to 2.48 before, during and after HSC respectively. Fear levels prior to HSC at 4.28 for VR patients and 3.52 for controls did not increase significantly during HSC in both groups: 4.44 vs 4.17. During HSC, pain levels increased from 1.40 to 4.720 in the VR group vs 0.65 to 4.109 (NS) in the controls, to decrease again afterwards to 2.60 vs 2.17 (NS) respectively.

Physicians rated the average pain levels of VR patients as 3.32 compared to 3.0 for controls, which was significantly correlated to patients' perception (p<0,005). Patients gave a positive rating to the VR experience (satisfaction score 7.17).

Limitations, reasons for caution: These are preliminary results, evaluating only a fourth of the required sample. A population selection bias could exist, as recruited patients were willing to accept VR. The very short induction period of 2 minutes could influence the effect of (immersiveness into) VR.
Wider implications of the findings: Pain management in ambulatory procedures should be multimodal and should include both pharmacological and non-pharmacological interventions. Introducing VR might increase patient tolerance for longer or more painful procedures. Offering a range of options will increase the spectrum of successful procedures *in the outpatient setting* and improve patient experience.
Trial registration number: B.U.N. 1432020000050

POSTER DISCUSSION

SESSION 76: STEM CELLS POSTER DISCUSSIONS

1 July 2021 Stream 3 11:45 - 12:45

P-796 Trial of Autologous Marrow derived Stem Cell Ovarian Transplantation (TAMSCOT) in young infertile women with diminished ovarian reserve for ovarian rejuvenation – HOPE still persists

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Study question: Does autologous bone marrow derived stem cell (BMDSC) ovarian transplantation optimize ovarian reserve parameters in young infertile women with diminished ovarian reserve (DOR) ?
Summary answer: The autologous stem cell ovarian transplantation (ASCOT) improves AFC and AMH by facilitating the recruitment of existing dormant follicles in young women with DOR.
What is known already: Oocyte donation is the practical therapeutic option when patients with premature ovarian ageing desire pregnancy. It involves significant psychological burden in terms of not able to have their own biological child. ASCOT has opened new doors in poor responders and premature ovarian insufficiency through its beneficial effects on ovarian reserve and IVF outcomes. However recent studies have shown contradictory results in terms of its efficacy. No prior study has been contemplated in DOR group
Study design, size, duration: An open label non randomized controlled trial was conducted at Division of Reproductive Medicine in collaboration with stem cell facility at tertiary care institute. Forty two infertile women less than 35 years age with DOR (AFC<5, AMH<1.2ng/ml and /or high FSH>8IU/l) were enrolled in the study during a period from January 2020 to December 2020. 20 women who did not opt for the intervention were treated as control group whereas 22 women received the intervention.
Participants/materials, setting, methods: Baseline hormonal profile (Day 2 FSH, estradiol, AMH and AFC) was done in all patients. Women with abnormal uterine cavity, endometriosis, prior ovarian surgery, abnormal karyotype were excluded. Bone marrow aspiration followed by mesenchymal stem cells isolation was performed. The stem cells were transplanted in both the ovaries through transvaginal route on the same day. Follow up visits were planned at one and six months to assess ovarian reserve parameters.
Main results and the role of chance: The mean age, BMI and duration of infertility were comparable between the control and study group (29.5±3.34vs 29.36±2.95years, 21.51±1.40vs21.87±1.93kg/m2, 6.9±1.94vs7.04±3.67 years). The positive response in terms of improved AMH and AFC was seen in 68% (15/22) patients. The mean number of stem cells injected in these women were 77.71±25.33 million. At first follow up, there was no significant difference between mean FSH, estradiol levels and mean right and left ovarian volume (9.23±3.95 vs 9.02±3.92mIU/l, 61.46±29.25 vs 68.12±62.52 pg/ml, 2.82±2.18 vs 2.44±1.25 cc, 2.02±1.54 vs 2.72±1.06 cc, p<0.05). There was significant increase in AMH and AFC values as compared to baseline (0.79±0.43 vs 1.26±0.82ng/ml, p=0.03; 3.47±1.30 vs 6.40±2.23, p<0.001). At second follow up visit, the significant increase in ovarian reserve persisted for AMH and AFC (0.79±0.43 vs 1.22±0.76 ng/ml, p=0.02; 3.47±1.30 vs 6.93±1.71,p<0.001). There was no significant difference between serum FSH , Estradiol and ovarian volume. None of the patients developed any complication and the improvement in AFC and AMH persisted during 10 month follow up period.
Limitations, reasons for caution: The limitation of present study is small sample size and non randomization. However, time period for which positive effect lasts has not been documented in earlier studies. This study is currently being endeavored, and women with improved ovarian reserve are followed up for any spontaneous conception or following assisted reproduction.
Wider implications of the findings: The present study demonstrates beneficial role of stem cells in improving ovarian reserve parameters in women with DOR with no acquired cause. If supported by future randomized clinical studies, it could represent a paradigm shift for fertility treatment in these women providing an opportunity to have their own biological child
Trial registration number: CTRI/2020/01/022726

P-797 A novel method for establishing human embryonic stem cells independent of feeder cells

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Study question: Is there a efficient establishing method of human embryonic stem cells directly from the human blastocysts independent of feeder cells?
Summary answer: We established a novel method of generating human embryonic stem cells directly from human blastocysts independent of feeder layer cells.
What is known already: Establishing embryonic stem cells lines mainly needed to coculture ICM clumps with feeder cells (like mouse or human fibroblasts) ,this brought in potential heterogeneous pollution.Although there had be some reports about generating human ESCs independent of feeder cells,but the efficiency was low and conditioned medium were unstable and also had the biological contamination.
Study design, size, duration: We used ten day5/6 donated human blastocysts from our reproductive center ,most of them were genetically diseased embryos with abnormal PGT diagnosis.After establishing ESCs procedure , all the cell lines were identified with pluripotency and differentiation potential tests. The success rate of system was calculated and compared with the conventional methods.
Participants/materials, setting, methods: In brief, ICM clumps were separated mechanically by using a micromanipulation system,and then transferred to a 30ul mTESR plus culture media drop pretreated with the geltrex (1:100 dilution) matrix and oxygen concentration was 5%. When cells attached and migrated,we also used laser to destroy the remaining trophoblast cells.About 10 days,the typical ES clone can be mechanically passaged and cells can be cultured in normal oxygen concentrations after passage 2..
Main results and the role of chance: Using this method we had successfully established nine embryonic stem cell lines from donated human blastocysts ,the success rate was 90% (9/10). Each cell lines had passed the evaluation test of embryonic stem cell. When compared with the conventional feeder cells dependent method,our novol methods not only eliminated the pollution from heterogeneous cells,but also had higher success rate (90% vs 25%).
Limitations, reasons for caution: Due to the scarcity of donated human blastocysts, this experiment was a single-center experiment with small samples.
Wider implications of the findings: We speculated that the batch differences of culture dishes, matrix and culture medium might affect the establish efficiency , and how to carry out a high level of quality control work might be the key factor to keep the system stable.
Trial registration number: basic research

P-798 Fertility preservation in pre-pubertal boys with cancer: A three-dimensional prepubertal testicular organoid for in vitro spermatogonial stem cell propagation and spermatogenesis

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Study question: Can a three-dimensional (3D) prepubertal testicular organoid be formed and provide an *in vitro* microenvironment for spermatogonial stem cells (SSCs) maintenance and future spermatogenesis?
Summary answer: Primary cells extracted from immature testicular tissue (ITT) or SSCs can be grown long-term as 3D organoids, providing the potential for *in vitro* study.
What is known already: Aggressive cancer treatments, such as chemo- or radiotherapy, can leave young prepubertal boys infertile. Such patients are recommended to undergo the cryopreservation of testicular material to protect future fertility. Within the testes, the specialized 3D structure and direct cell-to-cell interactions play a critical role in the proliferation and development of SSCs. Over recent decades, 3D culture systems and organoids have been used to culture cells *in vitro*, however, a system that allows investigations into testicular organogenesis *in vitro*, and its impact on the SSC niche, has yet to be developed.
Study design, size, duration: This study aims to develop a 3D organoid culture system to support the proliferation of SSCs and spermatogenesis. Primary bovine ITT cells and enriched SSCs were isolated and 3D organoids were generated by *in vitro* culture for up to 40 days. Organoid formation was observed after using different foundation cells seeded at different densities and cultured in medium containing gonadotropic supplements.
Participants/materials, setting, methods: Post-thaw bovine ITTs (2 weeks-of-age) were dissociated using two-step enzymatic digestion. Enriched SSCs were selected by Percoll gradients and differential plating. Viability and apoptosis were evaluated by trypan blue staining and TUNEL assays, respectively. SSCs were evaluated immunocytochemically for germ-cell markers (PGP-9.5, PLZF) and Sertoli cell markers (Vimentin, Sox9). Expression levels of SSCs and spermatogenesis-related genes (*Plzf, Gfrα-1, Nanog, Oct4, Stra8, Thy1*) were determined by real-time quantitative polymerase chain reaction (RT-qPCR).
Main results and the role of chance: The viability of digested cells from thawed ITTs was 78.667% ± 2.03. Total testicular cells (<10% SSCs) and enriched SSCs(>50% SSCs) were observed to self-assemble into structurally complex organoids recapitulating the cell type compartmentalization of the testis, in a 3D Matrigel-based culture system with 10% knockout serum replacement (KSR) culture medium, but not with 10% fetal bovine serum(FBS) medium. Testicular organoids were found to exhibit either a grape-like structure and a round-shape structure. Cytoplasmic extensions of spermatogonia/Sertoli cells were in contact with each other within a forming colony. Organoids were formed faster and larger when seeded at a final concentration of 1.5 × 106 cells/ml, compared to 5 × 105 cells/ml and 1.5 × 105 cells/ml. Organoids grew to a diameter of 400 µm within 10 - 15 days and were passaged by mechanical disruption at a ratio of 1:3 every 7 - 10 days. Immunocytochemistry results showed that clusters of PGP9.5 and PLZF-positive cells were present within the organoids. The expression of selected germ cell and spermatogenesis markers in the testicular organoids closely resembled that of primary testicular cells for up to 20 days of culture.
Limitations, reasons for caution: We used calves (2 weeks-of-age) as an animal model to study testicular organoids. This tissue may act differently than human tissues and may not fully represent prepuberty. Furthermore, we only evaluated gene expression levels for selected markers that may not represent the full functional capability of germ cells.
Wider implications of the findings: Testicular organoids, as an *in vitro* bioengineering testicular model, could potentially be used to study testicular tissue development, cellular interactions, endocrinology, and spermatogenesis, in the laboratory but may also be applied for clinical purposes in the future.
Trial registration number: University of Oxford

P-805 Artificial oocytes: from somatic cells to fertile pups

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Study question: We analyzed the efficacy of generating artificial oocytes using somatic cells (SCs) from two mouse strains (B6D2F1 and FVB) and followed their full pre-/post-implantation development.
Summary answer: While artificial oocytes generated from the new strain (FVB) had higher fertilization rates, those from the standard strain (B6D2F1) provided expanded blastocysts and fertile pups.
What is known already: B6D2F1 is a popular hybrid mouse strain for cloning and transgenic creation due to its geno-/pheno-typic uniformity and high oocyte yield and quality. Indeed, B6D2F1 oocytes have a distinct metaphase II (MII) spindle complex, making them an ideal candidate to generate ooplasts used in SC nuclear transfer (SCNT). However, because they lack genetic variance, they are less suitable for reciprocal SCNT studies. In contrast, FVB mice have single nucleotide polymorphisms and indels on each chromosome that can aid in tracing the pedigree of progeny.
Study design, size, duration: A total of 10 experiments were performed over the course of 3 months, using 30 stimulated mice. SCs were retrieved from cumulus oophorus harvested from FVB and B6D2F1 mice. SCs from both strains were injected into enucleated MII B6D2F1 oocytes. Unmanipulated B6D2F1 oocytes were piezo-ICSI inseminated, serving as controls. The occurrence of haploidization, fertilization, and full preimplantation development was compared. Some blastocysts were transferred into pseudo-pregnant CD-1 mice to obtain offspring.
Participants/materials, setting, methods: Oocyte enucleation was performed under Oosight™ visualization and cytochalasin B exposure. An FVB or B6D2F1 SC was transferred into the perivitelline space of the ooplast with Sendai virus to promote fusion. Haploidization was monitored by pseudo-meiotic spindle formation followed by extrusion of a pseudo-polar body after insemination. Conceptuses were cultured in a time-lapse imaging system, with piezo-ICSI controls. Expanded blastocysts were transferred into uterine horns of pseudo-pregnant mice. Offspring were mated to test their fertility.
Main results and the role of chance: FVB (n=278) and B6D2F1 (n=905) SCs at G0 phase, with a diameter <10 mm, were chosen for SCNT and transferred into enucleated B6D2F1 ooplasts. Enucleation of 1,212 oocytes yielded a survival rate of 97.6%. Both FVB and B6D2F1 SCNT resulted in similar survival rates of 100% and 98.5%, respectively. Successful haploidization, determined by the presence of a pseudo-meiotic spindle 2 hours after SCNT, was also comparable, with 59.9% of FVB and 63.7% of B6D2F1. Survival after piezo-ICSI was also comparable between FVB- and B6D2F1-reconstituted oocytes, with rates of 64.3% and 60.3%, respectively, albeit lower than the control (75.2%, $P<0.00001$). FVB embryos fertilized at a rate of 88.7%, comparable to the control zygotes at 85.8%, while B6D2F1 conceptuses demonstrated a lower fertilization rate (70.8%, $P<0.00001$). Blastulation of FVB- and B6D2F1-derived embryos was 15.1% and 24.0%, respectively, while the control was 80.7% ($P<0.00001$). Whole-genome karyotyping of 9 B6D2F1-derived blastocysts confirmed 5 of the samples to be euploid. FVB blastocysts (N=8) and B6D2F1 blastocysts (N=81) were transferred into pseudo-pregnant mice, resulting in 3 fertile offspring only from the B6D2F1 conceptuses.
Limitations, reasons for caution: This is still a limited number of observations, and pups were delivered only from the B6D2F1 strain. The utilization of a strain with higher genetic variance may help facilitate offspring fingerprinting.
Wider implications of the findings: This study demonstrates the ability to generate artificial genotyped conceptuses, yielding live offspring. The identification of a feasible donor cell, together with optimization of cell cycle stage and standardization of post-implantation development, will help promote this technique for human reproduction in couples with age-related infertility or poor ovarian reserve.
Trial registration number: N/A

INVITED SESSION

SESSION 77: DEMOGRAPHIC IMPACT OF ART

01 July 2021 Stream 4 11:45 - 12:45

O-069 A demographic revolution? Social representations confronted with statistics

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Abstract text

Assisted reproductive technologies (ART) regularly hit the media. Most people have an idea of ART that is based only on this prism. This restrictive view may

lead to major discrepancy between what the general population thinks of these treatments and the everyday reality of ART. The most striking example of this discrepancy is probably the use of third party donors (sperm, oocyte, embryo or gestational donation). In France, the media focus almost exclusively on ART with a third party donor. The personalities who relate their experience in the media or in autobiographies are all children (now adults or adolescents) who were conceived with a third-party donor. Nevertheless, 95% of children conceived by ART in France have not been conceived through a third party. The media also highlight exceptional individual stories that give rise to strong societal controversies, such as Natalie Suleman (USA) who was called "Octomom" after she gave birth to octoplets, or Maria del Carmen Bousada de Lara (Spain) and Adriana Iliescu (Romania) who gave birth at age 66, or more recently Lulu and Nana (China) who were genetically modified twin sisters. Such reports can arouse wonder or fear, but both lead to a social representation of ART as an "omnipotent" technique. Infertile couples whose knowledge of ART is based on the media coverage may venture into these treatments thinking that as their case is an "ordinary" one, there should be no problem for them in having a baby through these technologies.

Clinical statistics on ART show that even if the success rate is high, there is a gap between social expectations and reality. These statistics can be misleading, as they often assume that the couple has undergone several ART cycles. The objective of clinical statistics is usually to measure the efficacy of ART from a medical viewpoint, not from the standpoint of the couples' care pathways. The gap between the two is considerable. The pathways of couples who undertake ART are marked by pitfalls that strongly affect success rates because of the risk of treatment dropout. In some countries, economic factors are a major reason for dropout because of the high cost of ART. France is a very interesting textbook case to explore this issue, as all infertility treatments are fully reimbursed for up to six artificial inseminations and four in vitro fertilizations for each birth. Economic barriers to ART access are minimal in such a favorable national context. Nevertheless, only about half of couples treated by ART finally become parents and success rates drop dramatically in older women. This epidemiological statistical reality is difficult to reconcile with the media presentation of ART as "omnipotent". However, "natural miracles" can also occur as spontaneous births have been observed among couples unsuccessfully treated by ART. There are also other pathways to parenthood, such as adoption of a child.

Thanks to ART, every year numerous couples become parents. But for infertile couples, the everyday reality is far from the "omnipotence" acclaimed by media headlines. The social representation of ART must move toward a more balanced perception of these technologies, bearing in mind its successes and also its limitations, especially with the current demographic trend towards childbearing at a later age that may lead to an increase in demand for ART. Change in the social representation of ART will probably need to go far beyond classic public health campaigns. ART will need to be approached differently in cultural spaces such as the media but also in movies, series or novels that have a major influence on collective social imaginaries and representations.

O-070 Demographic impact of increased ART use in European countries

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SELECTED ORAL COMMUNICATIONS

SESSION 78: RECENT DEVELOPMENTS IN EMBRYO SELECTION

01 July 2021 Stream 1 14:00 - 15:15

O-219 Detailed morpho-kinetic analysis of the first cleavage can help in evaluating the viability of direct-cleaved human zygotes

T. Shimura[1], **K. Yumoto**[1], **M. Sugishima**[1], **Y. Mio**[1]

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Study question: Why do some direct-cleaved human zygotes still lead to a live birth?

Summary answer: Direct-cleaved zygotes which have undergone the 2-cell stage can lead to a live birth, while zygotes cleaved from 1-cell to ≥3-cell do not.

What is known already: In recent years, zygotes that develop from 2-cell to 3-cell within 5 hours after the first cleavage have been evaluated as "direct-cleaved" zygotes, because normal cleavage takes approximately 12 hours to complete. It was reported that their implantation rate was significantly lower than zygotes with normal cleavage pattern, and eliminating direct-cleaved zygotes from transfer could improve the implantation rate. However, some direct-cleaved zygotes at the first cleavage could still lead to a live birth. Few reports have examined the difference between a cleavage from 1-cell to ≥3-cell and 2-cell to ≥3-cell within 5 hours after the first cleavage.

Study design, size, duration: A retrospective study involving 2,077 cycles of IVF/ICSI between July 2012 and July 2019. A total of 5,991 normally fertilized zygotes (2PN/2PB) were included. Of those, 3,508 were evaluated as usable good/fair quality embryos on Day2/3, and the rest (n=2,483) were evaluated as poor quality and rejected from transfer or cryopreservation after 7 days of culture. Of 3,508 usable embryos, 884 were selected based on the availability of results of live birth for this study.

Participants/materials, setting, methods: Time-lapse imaging (5 slices along Z-axis every 10 minutes) was performed in EmbryoScopeTM. Zygotes were morphokinetically analyzed in detail and classified into four groups by their cleavage patterns: Group1 (1-cell→2-cell); Group 2 (1-cell→3-cell); Group 3 (1-cell→2-cell→≥3-cell within 5 hours after the first cleavage); and Group 4 (1-cell→2-cell→≥5-cell). The proportion, mean maternal age and live birth rate of each group were examined.

Main results and the role of chance: The proportion of Groups 1-4 was 83.6% (n=739), 3.8% (n=34), 5.9% (n=52), and 6.7% (n=59), respectively. 0f 884 zygotes examined in this study, the mean maternal age was significantly higher in Group 2 and 4 than in Group 1 ($P < 0.05$; 37.4±4.9 in Group1, 39.1±5.2 in Group 2, 38.6±6.0 in Group 3, and 38.7±5.1 in Group 4). The rate of confirmed gestational sac was significantly lower in Group 2 and 4 than in Group 1 [$P < 0.01$; 36.3% (n=268/739), 0% (n=0/34), 25.0% (n=13/52), and 18.6% (n=11/59) in Groups 1-4, respectively]. Furthermore, the live birth rate was significantly higher in Group 1 than in Groups 2, 3 and 4 [$P < 0.01$; 28.4% (n=210/739), 0% (n=0/34), 13.5% (n=7/52), and 15.3% (n=9/59) in Groups 1-4, respectively]. Above all, while zygotes in Group 2 showed no pregnancy and live birth at all, zygotes in Group 3 showed a live birth rate of 13.5%. However, they had a significantly higher miscarriage rate (42.9%, n=6) compared to zygotes in Group 1 (19.5%, n=55).

Limitations, reasons for caution: It is very difficult to capture cleavage patterns by routine observations because the timings of developmental events are different between embryos. A time-lapse imaging and culturing system is essential to solve this problem, however, it cannot visualize the distribution of chromosomes, and no chromosomal analysis was conducted in this study.

Wider implications of the findings: This study revealed that zygotes previously classified as "direct-cleaved" and eliminated from transfer included viable zygotes which could lead to a live birth. Therefore, it is crucial to optimize the use of time-lapse imaging of human zygotes in order to precisely evaluate the first cleavage.

Trial registration number: not applicable

O-220 An annotation-free embryo scoring system (iDAScore®) based on deep learning shows high performance for pregnancy prediction after single-vitrified blastocyst transfer

S. Ueno[1], **M. Ito**[1], **K. Uchiyama**[1], **T. Okimura**[1], **A. Yabuuchi**[2], **K. Kato**[3]

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Study question: How is the performance of an automated embryo scoring system for pregnancy prediction after single-vitrified blastocyst transfer (SVBT) compared to other, annotation-dependent blastocyst grading systems?

Summary answer: Automatic embryo ranking by iDAScore shows a higher or equal performance, with regards to pregnancy prediction after SVBT, compared to manual, annotation-dependent grading systems.

What is known already: Blastocyst viability can be assessed by blastocyst morphology grades and/or morphokinetic parameters. However, morphological and morphokinetic embryo assessment is prone to both inter- and intra-observer variation. Recently, embryo ranking models have been developed based on artificial intelligence (AI) and deep learning. Such models rank embryos according to their potential for pregnancy only based on images and do not require any user-dependent annotation. So far, no study has independently assessed the performance of AI models compared to other embryo scoring models, including traditional morphological grading.
Study design, size, duration: A total of 3,014 SVBT cycles were retrospectively analysed. Embryos were stratified according to SART age groups. The quality and scoring of embryos were assessed by iDAScore v1.0 (iDAS, Vitrolife, Sweden), KIDScore™ D5 v3 (KS; Vitrolife), and Gardner criteria. The performance of the pregnancy prediction for each embryo scoring model was compared using the area under curve (AUC) of the receiver operating characteristic curve for each maternal age group.
Participants/materials, setting, methods: Embryos were cultured in the EmbryoScope+ and EmbryoScopeFlex (Vitrolife). iDAS was automatically calculated using the iDAScore model running on the EmbryoViewer (Vitrolife). KS was calculated in EmbryoViewer after annotation of the required parameters. ICM and TE were annotated according to the Gardner criteria. The degree of expansion in all blastocysts was Grade 4 due to our freezing policy. Furthermore, Gardner's scores were stratified into four grades (Excellent: AA, Good: AB BA, Fair: BB, Poor: others).
Main results and the role of chance: The AUCs of the < 35 years age group (n = 389) for pregnancy prediction were 0.72 for iDAS, 0.66 for KS and 0.64 for Gardner criteria. The AUC of iDAS was significantly higher (P< 0.05) compared to the other two models. For the 35–37 years age group (n = 514) the AUCs were 0.68, 0.68, and 0.65 for iDAS, KS and Gardner, respectively, and were not significantly different. The AUCs of the 38–40 years age group (n = 796) were 0.67 for iDAS, 0.65 for KS and 0.64 for Gardner criteria and where was not significantly different. The AUCs of the 41–42 years age group (n = 636) were 0.66, 0.66, and 0.63 for iDAS, KS and Gardner, respectively, and there was no significant difference among the pregnancy prediction models. For the > 42 years age group (n = 389) AUCs were 0.76 for iDAS, 0.75 for KS and 0.75 for Gardner criteria and not significantly different. Thus, for all age groups, iDAS was either highest or equal to the highest AUC, although a significant difference was only observed for the youngest age group.
Limitations, reasons for caution: In this study, SVBT was performed after minimal stimulation and natural cycle in vitro fertilisation (IVF). Therefore, we had only few cycles with elective blastocyst transfer. However, there was also no bias in selecting the embryos for SVBT.
Wider implications of the findings: Our results showed that objective embryo assessment by a completely automatic and annotation-free model, iDAScore, does perform as good or even better than more traditional embryo assessment or an annotation-dependent ranking tool. iDAS could be an optimal pregnancy prediction model after SVBT, especially in young and advanced age patients.
Trial registration number: not applicable

O-221 Stage and morphology of the competent blastocyst are associated with pregnancy and birth outcomes; a multicenter cohort study

M.L. Groendahl[1], M. Buhl Borgstrøm[2], U. Schiøler Kesmodel[3]

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Study question: Do stage and morphology of the competent blastocyst associate with initial hCG rise, gestational age, preterm birth, child birth weight, length, and child sex?
Summary answer: Higher stage, TE- and ICM-scores associated with higher hCG-rise; ICM- and TE-scores associated with length at birth, and higher stage and TE-score associated with boys.
What is known already: Many studies have focused on the developmental stage and morphology of the blastocysts in order to find biomarkers of competence to improve the efficacy of assisted reproduction technology treatment. In contrast, the associations between blastocyst assessment score parameters (individually or by combined score) and perinatal outcome have only been reported in few and smaller single center studies, and conflicting results have been presented. In the present study, we focused on the in vitro cultured blastocyst leading to a live birth and how the stage and morphology of these competent blastocysts relate to implantation and birth outcomes.
Study design, size, duration: Multicenter historical cohort study based on exposure (blastocyst stage (1-6) and morphology (trophectoderm (TE) and inner cell mass (ICM): A,B,C)) and outcome data (serum human chorionic gonadotrophin (hCG), gestational age, preterm birth, child weight, length, and sex) from women undergoing single blastocyst transfer resulting in singleton pregnancy and birth. Data from 16 private and university-based facilities for clinical services and research from 2014 to 2018 was included.
Participants/materials, setting, methods: 7246 women, who underwent ovarian stimulation or Frozen-thawed-Embryo-Transfer with single blastocyst transfer resulting in singleton pregnancy were identified. Linking to the Danish Medical Birth Registry resulted in a total of 4842 women with live birth being included. Initial serum hCG value (IU/L) (11 days after transfer), gestational age (days), preterm birth (%) child weight (grams), length (cm) and sex. The analyses were adjusted for female age, BMI, smoking, center, diagnosis, parity, gestational age and sex.
Main results and the role of chance: Higher mean initial hCG was consistently positively associated with higher developmental stage (p<0.001), TE (p<0.001) and ICM score (p=0.02); for stage 6, TE (A) and ICM (A): 508.4, 436.5 and 428.5 IU/L, respectively. No differences between blastocyst morphology (stage, TE, ICM), gestational age (mean 276.6 days), preterm birth (8.3%) and birth weight (mean 3461.7 gram) were statistically significant. While stage showed no association with length at birth (mean 51.6 cm), length at birth between blastocysts with a TE score C and a TE score A were statistically significant (mean difference 0.5 cm (0.07;0.83)) as was the length at birth between blastocysts with an ICM score B and C compared to score A, mean differences respectively 0.2 cm (0.02;0.31) and 0.5 cm (0.03;0.87). Stage and TE, but not ICM were associated with the sex of the child. Blastocysts transferred with stage score 5 compared to blastocysts transferred with score 3 had a 33% increased probability of being a boy (OR 1.33 (1.08;1.64)). Further, TE score B blastocysts compared to TE score A blastocysts had a 28% reduced probability of being a boy (OR 0.72 (0.62;0.82)).
Limitations, reasons for caution: The assessment scores of the blastocysts´ stage and morphology were based on subjective evaluation, and information bias may have influenced the results. By adjusting for center, we took the potential variation in scoring between clinics into considerations.
Wider implications of the findings: Stage and morphology of the competent blastocyst was associated with initial hCG rise suggesting an effect on implantation, which may be used in routine, everyday information to women and couples on the day of blastocyst transfer.
Trial registration number: j.nr.: VD-2018-282

O-222 An artificial intelligence model that was trained on pregnancy outcomes for embryo viability assessment is highly correlated with Gardner Score

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Study question: Do artificial intelligence (AI) models used to assess embryo viability (based on pregnancy outcomes) also correlate with known embryo quality measures such as Gardner score?
Summary answer: An AI for embryo viability assessment also correlated with Gardner score, further substantiating the use of AI for assessment and selection of good quality embryos.
What is known already: The Gardner score consists of three separate components of embryo morphology that are graded individually, then combined to give a final score describing Day 5 embryo (blastocyst) quality. Evidence suggests

the Gardner score has some correlation with clinical pregnancy. We hypothesized that an AI model trained to evaluate likelihood of clinical pregnancy based on fetal heartbeat (in clinical use globally) would also correlate with components of the Gardner score itself. We also compared the ability of the AI and Gardner score to predict pregnancy outcomes.

Study design, size, duration: This study involved analysis of a prospectively collected dataset of single static Day 5 embryo images with associated Gardner scores and AI viability scores. The dataset comprised time-lapse images of 1,485 embryos (EmbryoScope) from 638 patients treated at a single in vitro fertilization (IVF) clinic between November 2019 and December 2020. The AI model was not trained on data from this clinic.

Participants/materials, setting, methods: Average patient age was 35.4 years. Embryologists manually graded each embryo using the Gardner method, then subsequently used the AI to obtain a score between 0 (predicted non-viable, unlikely to lead to a pregnancy) and 10 (predicted viable, likely to lead to a pregnancy). Correlation between the AI viability score and Gardner score was then assessed.

Main results and the role of chance: The average AI score was significantly correlated with the three components of the Gardner score: expansion grade, inner cell mass (ICM) grade, and trophectoderm grade. Average AI score generally increased with advancing blastocyst developmental stage.

Blastocysts with expansion grades of ≥3 are generally considered suitable for transfer. This study showed that embryos with expansion grade 3 had lower AI scores than those with grades 4-6, consistent with a reduced pregnancy rate. AI correlation with trophectoderm grade was more significant than with ICM grade, consistent with studies demonstrating that trophectoderm grade is more important than ICM in determining clinical pregnancy likelihood.

The AI predicted Gardner scores of ≥2BB with an accuracy of 71.7% (sensitivity 75.1%, specificity 45.9%), and an AUC of 0.68. However, when used to predict pregnancy outcome, the AI performed 27.9% better than the Gardner score (accuracies of 49.8% and 39.0% respectively).

Even though the AI was highly correlated with the Gardner score, the improved efficacy for predicting pregnancy suggests that a) the AI provides an advantage in standardization of scoring over the manual and subjective Gardner method, and b) the AI is likely identifying and evaluating morphological features of embryo quality that are not captured by the Gardner method.

Limitations, reasons for caution: The Gardner score is not a linear score, creating challenges with setting a suitable threshold relating to the prediction of pregnancy. The 2BB treshold was chosen based on literature (Munné et al 2019) and verified by experienced embryologists. This correlative study may also require additional confirmatory studies on independent datasets.

Wider implications of the findings: The correlation between AI scores and known features of embryo quality (Gardner score) substantiates the use of the AI for embryo assessment. The AI score provides further insight into components of the Gardner score, and may detect morphological features related to clinical pregnancy beyond those evaluated by the Gardner method.

Trial registration number: Not applicable

O-223 Fatty acid supplementation into warming solutions improve the developmental competence of mouse, bovine, and human oocytes and embryos after vitrification

K. Ohata[1], K. Ezoe[1], T. Miki[1], S. Kouraba[2], N. Fujiwara[1], A. Yabuuchi[1], K. Kato[3]

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Study question: Does fatty acid (FA) supplementation into vitrification and warming solutions influence the developmental competence of oocyte and embryo after vitrification and warming?

Summary answer: FA supplementation during the warming process improves the developmental competence of vitrified-warmed mouse oocytes and embryonic-morphologies after vitrification at the cleavage-stage in bovines and humans.

What is known already: Vitrified metaphase II stage oocytes exhibit a diminished ability to develop into blastocysts and live births. Previous studies have shown reduction in intracellular lipid content as one of the factors associated with reduced developmental competence of oocytes after vitrification as the intracellular lipid content of oocytes is affected by vitrification. FAs derived from break down of lipids are primarily transferred to the mitochondria, where it plays a crucial role in cellular metabolism. However, the effects of FA supplementation in warming solutions on the cytoplasmic lipid content and subsequent embryo development are unknown.

Study design, size, duration: A chemically defined FA mixture was added to the vitrification and/or warming solutions. Oocytes collected from C57BL6/N (n=80) were randomly divided into three groups (fresh, n=634; non-FA (control), n=961; FA, n=1,686), and were vitrified-warmed with/without FA. Lipid composition, developmental competence, and gene expression levels were compared among the groups. Bovine embryos (fresh, n=420; control, n=524; FA, n=492) and discarded human day-2 embryos (control, n=87; FA, n=92) were used to examine the developmental competence of embryos.

Participants/materials, setting, methods: Lipids in the ooplasm were stained with Nile red and the fluorescence intensity was analysed. The developmental competence of mouse oocytes was examined by performing intracytoplasmic sperm injection. Expressions of FA metabolism-related genes were measured. The bovine embryos were vitrified at the four-cell stage and cultured to the blastocyst stage after warming. Cryopreserved discarded human embryos were warmed and cultured. The obtained blastocysts were then placed on fibronectin-coated dishes to examine the outgrowth formation.

Main results and the role of chance: Lipid content of mouse oocytes was significantly lower in the control group compared to that in the fresh group ($P<0.05$). On the contrary, lipid contents of FA and fresh groups were comparable ($P=0.24$). Blastocyst formation rate was significantly higher in the FA group than that in the control group (55.7% and 44.8%, respectively; $P<0.05$). To examine the optimal timing for FA supplementation, FA was added to the vitrification solution (FAvit), warming solution (FAthaw), and/or both solutions (FAvit-thaw). Blastocyst formation rate was significantly higher in the FAthaw group than that in the control group (59.8% and 50.0%, respectively; $P<0.05$). The mRNA expressions of *Acaa2* and *Hadha* in mouse embryos were significantly higher in the FAthaw group compared to that in the control group ($P<0.05$). Moreover, FA supplemented warming solutions significantly improved the blastocyst formation rate in bovines (control, 53.5%; FAthaw, 64.5%; $P<0.05$). Developmental rate to the expanded blastocyst stage was slightly improved in human embryos (control, 53.7%; FAthaw, 63%; $P=0.38$) and the proportion of Grade A in inner cell mass and trophectoderm was significantly higher in the FAthaw group than that in the control group ($P<0.05$). There were no differences in the outgrowth abilities between the control and FAthaw groups.

Limitations, reasons for caution: Since the experiments of the current study on human embryos were performed *in vitro* using discarded embryos, in vivo developmental ability was not evaluated. Therefore, to validate the application of our findings in human assisted reproductive technologies, further clinical trials (ART) are warranted.

Wider implications of the findings: FA supplementation into the warming solutions improved the developmental competence of vitrified–warmed oocytes and cleaved embryos by activating the β-oxidation pathway. These results indicate that FA supplementation into warming solutions is a potential strategy to improve clinical outcomes in human ART.

Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 79: AGE, DISEASE, AND THEIR IMPACT ON MALE FERTILITY

01 July 2021 Stream 2 14:00 - 15:15

O-224 Paternal ageing impacts blastulation and pregnancy outcomes at different levels of maternal age: a clustering analysis of 21,960 injected oocytes and 3837 ICSI cycles

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Study question: Are the morphological parameters and development of in vitro cultured embryos, and intracytoplasmic sperm injection (ICSI) outcomes influenced by maternal and paternal ageing?
Summary answer: The slopes of maternal age on blastulation, blastocyst quality, and implantation, pregnancy and miscarriage rates significantly changed (worsened) for every year increase in paternal age.
What is known already: Due to the vast literature demonstrating that female age interferes with intracytoplasmic sperm injection (ICSI) outcomes, there is an imposition, in numerous countries, regarding maternal age limit for assisted reproduction. Despite several studies have underscored the negative impact of paternal age and lifestyle factors on reproductive health, the influence of paternal age on ICSI outcomes is still a matter of debate. The aim of this study was to investigate if the effect of paternal age on embryo development differs at different values of maternal age, thus creating a rationale for the data to reach physicians, patients, and public health recommendations.
Study design, size, duration: This historical cohort study included 3837 couples undergoing their first ICSI cycle from January/2014 to October/2020. A total of 21960 oocytes were injected and embryos were evaluated until day 5 of development. The main effects of maternal and paternal ages, as well as the effect of their product (interaction term) on embryo growth and development, and on pregnancy outcomes were investigated taking into account clustering of data (multiple embryos per cycle), using generalized mixed models.
Participants/materials, setting, methods: The study was performed in a private university-affiliated in vitro fertilization center. Zygotes were morphologically evaluated 17h post ICSI. For days 2 and 3 of development, the number of blastomeres, blastomere symmetry, percentage of fragmentation and presence of multinucleation were recorded. On day 5 of development, successful blastulation, and inner cell mass and trophectoderm qualities were recorded. Pregnancy was calculated per transfer, and miscarriage was defined as pregnancy loss before 20 weeks gestation.
Main results and the role of chance: The coefficients for the interaction term were statistically significant for blastocyst development (B: - 0.005, OR: 0.995, CI: 0.994 – 0.996, $p < 0.001$), top-quality blastocyst (B: - 0.003, OR: 0.997, CI: 0.996 – 0.999, $p < 0.001$), implantation rate (B: - 0.041, OR: 0.960, CI: 0.947 – 0.973, $p < 0.001$), pregnancy rate (B: - 0.004, OR: 0.996, CI: 0.995 – 0.997, $p < 0.001$), and miscarriage rate (B: 0.011, OR: 1.012, CI: 1.005 – 1.018, $p = 0.001$). These values describe the changes in slopes such that, the slope of one independent variable (e.g. maternal age) on the dependent variable (e.g. implantation rate) changes by the value of B (- 0.041) for every unit change on the other independent variable (e.g. paternal age). No significant results were observed for the influence of the interaction term on embryo morphological features on days 1, 2 and 3 of development. Two post hoc power analyses were calculated, given α of 5%, sample size of 21960 zygotes and 3315 ICSI cycles with embryo transfer and effect sizes for blastulation and pregnancy outcomes, respectively. The achieved power was superior to 99% in both analyses.
Limitations, reasons for caution: The retrospective and monocentric nature of the study are its major limitations.
Wider implications of the findings: Our results underscore the importance of both maternal and paternal ages for blastulation and successful pregnancy. Main effects of paternal and maternal ages should no longer be interpreted as the relationship between each independent variable and a given outcome, but rather be conditional on the values of the interaction term.
Trial registration number: Not applicable

O-225 Effects of SARS-Corona virus 2 on IVF treatment parameters: A cohort study of post COVID-19 patients

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Study question: Does recovery from SARS–Corona virus 2 (SARS–CoV-2) infection negatively effect IVF cycle parameters?
Summary answer: Female IVF treatment parameters were comparable to the pre-Covid-19 infection cycle performance. Sperm concentration and motility demonstrated lower mean counts following Covid-19 infection.
What is known already: Corona-virus disease-19 (Covid-19) is a global pandemic caused by SARS–Corona virus 2 (SARS–CoV-2). The virus primarily affects the respiratory system, but other systemic and immune mediated effects have been reported. The spikes of SARS-CoV-2 have strong affinity for the Angiotensin converting enzyme (ACE) 2 receptor, leading to an increased Angiotensin II (Ang II) mediated pro-inflammatory response. ACE2 receptors exist in the human reproductive tract (more in males) and pose a regulatory role together with Ang II. So far, reports have been inconsistent regarding testicular effects. Other implications involving fertility and fertility treatment post infection are scarce.
Study design, size, duration: In this retrospective cohort study, IVF cycle performance was compared before and after Corona-virus disease-19. Patients were included only in cases where an IVF cycle was initiated within 3 months of Covid-19 recovery, between March 2020-December 2020.
Participants/materials, setting, methods: The study was conducted in a University affiliated IVF unit. Post Covid- 19 cycle parameters were compared to previous cycles of the same individual prior to infection. If previous cycles were not available, parameters were compared to non-exposed patients of same age, same treatment and identical indication. Sperm concentration and motility were compared before and after infection. Non exposure was defined by a lack of past Covid-19 diagnosis and a negative PCR throughout the treatment.
Main results and the role of chance: All together, including the matched cycles, we compared 40 cycles which started within 3 months of recovery: 26 fresh stimulation cycles and 14 frozen thawed transfer cycles. In 28 of these cycles the patient could serve as its own control. Mean age for the female partner was 33.2 years ±6.5 years. Eight male partners presented post infection and provided fresh samples for a cycle involving fertilization. We compared stimulation parameters including maximal Estradiol level, stimulation length, FSH dosage, number of oocytes retrieved, fertilization rates, number of embryos created, high quality embryo number and endometrial thickness. All of these were comparable to non-exposed cycles (generalized estimating equations, p values >0.1). No complications were recorded, specifically no thromboembolic events or respiratory complications. A total of 8 patients conceived: 1 was a chemical pregnancy, 1 extra-uterine pregnancy, 3 miscarriages and 3 ongoing, of those 1 was complicated by early bleeding. Male sperm analyses showed a trend towards lower post disease parameters, not reaching a statistical significance: 23mil/ml compared to 13.6 and 20.7% progressive motility compared to 12.3% (p values 0.09 and 0.17, respectively).
Limitations, reasons for caution: Current results are based on a small sample size, still insufficient for deducing definite conclusions or guidelines. Pregnancy outcome following IVF treatment in Covid-19 recoverees should further be studied. By the time of the conference, the number of cases is expected to be significantly higher.
Wider implications of the findings: This study provides preliminary data regarding the effects of SARS-COV-2 infection on IVF treatment outcomes. Despite the small sample size, treatment parameters seem unaffected, however, sperm performance seems to be compromised. Health policy and patients' decisions regarding whether or not to postpone IVF procedures necessitates additional data.
Trial registration number: Not applicable - retrospective

O-226 The microscopic se men improvement after surgical varicocele repair

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Study question: Evaluation of the effect of varicocele correction on conventional and microscopic seminal parameters and evaluation of which factors might predict sperm improvement after surgical treatment.
Summary answer: The detailed morphologic sperm evaluation has been identified as a novel parameter expressing the post-surgical semen amelioration after varicocelectomy.
What is known already: Generally, varicocele develops during puberty and occurs more often on the left side for anatomic reasons. However, its diagnosis is commonly delayed, especially in asymptomatic cases, until the man consults an andrologist for couple infertility. A causative relationship between varicocele and impairment of semen quality has been largely investigated in the context of male infertility. Despite the florid literature available on this topic, the clinical benefit in terms of semen quality improvement after varicocele surgical repair remains controversial.
Study design, size, duration: An observational, retrospective clinical trial was carried out including patients undergoing surgical treatment for varicocele at the Day Surgery of the Urology Operative Unit of the Santa Maria Nuova Hospital-IRCCS of Reggio Emilia from September 2011 to March 2020. Primary outcome was the detailed morphologic microscopic sperm evaluation. Secondary outcomes were conventional semen analyses. Each patient was considered two times (before and after the surgery) and evaluated by both physical examination and ultrasonography.
Participants/materials, setting, methods: The inclusion criteria considered the patients with diagnosis of varicocele at ultrasound examination, attending surgical resolution and with at semen analyses before and after the surgery, were excluded patients with diagnosis of varicocele without surgical indication, and/or semen analyses performed only before or only after the surgery. In the statistical analysis a logistic multivariate regression analysis was performed in order to evaluate the change before and after surgery.
Main results and the role of chance: The post-surgical semen analysis was performed after a mean of 183.7 + 112.5 days since the surgery for a total of 121 males (mean age 24.6 + 4.1 years) enrolled. The surgical treatment leads to a significant increase in sperm concentration (p=0.015) and percentage of progressive and total motility (p=0.022 and p=0.039, respectively), with a significant decrease in the percentage of immobile sperms (p=0.013). In particular, semen concentration improved in 71.7% of patients (p=0.010). Considering the detailed morphologic microscopic evaluation, a significant improvement was detected: head abnormalities showed a significant reduction, considering microcephaly (3.3 + 3.6 versus 2.2 + 2.9%, p=0.015), macrocephaly (1.4 + 0.6 versus 1.2 + 0.9%, p=0.043) and cytoplasmic appendix (1.4 + 0.8 versus 0.9 + 1.2%, p=0.041). Moreover, surgery led to a significant reduction of tails abnormalities, considering absence (0.6 +2.3 versus 0.1 + 0.7, p=0.048) and coiled tail (5.2 + 1.5 versus 6.6 + 2.0, p=0.037). Thus, surgical varicocele resolution leads to a significant improvement in specific morphological semen parameters. The multivariate logistic analysis identified the ultrasound varicocele degree before surgery as a main predictor of the sperm concentration improvement (p=0.016). The semen parameters improvement was higher for varicocele of I and II degree (p=0.008).
Limitations, reasons for caution: The retrospective study design precluded from carrying out a case-control study to compare the surgical techniques. Moreover, the study design limited the availability of patients' clinical data in order to performed a more comprehensive predictive analysis.
Wider implications of the findings: Using a complex statistical approach, it emerged that the greatest improvement in semen quality was obtained in case of mild varicocele, increasing the knowledge on the therapeutic potential of surgery. This result has clinical implications, since it could help to select those patients 'to treat or not to treat'.
Trial registration number: none

O-227 Pre-treatment semen parameters in haematological malignancies. An analysis of sequential samples.

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Study question: Does Leukaemia affect spermatogenesis more adversely than Hodgkin's lymphoma and is the effect consistent in sequential samples?
Summary answer: LLeukaemia is associated with a higher incidence of azoospermia, oligozoospermia and asthenozoospermia compared to Hodgkin's lymphoma. These findings were consistent in sequential samples.
What is known already: Hodgkin's lymphoma (HL) and leukaemias are common haematological malignancies that affect young men. Although not all treatments for these malignancies are gonadotoxic, there is evidence that malignancy affects sperm quality. Our own analysis in over 3000 men revealed that a diverse group of malignancies affected semen parameters adversely. There is concern that a single sample analysis may not reveal the true state due to varied period of abstinence and naturally occurring variation in semen quality. Leukaemia and lymphoma are systemic diseases; leukaemia usually runs a more torrid course whilst HL a more indolent course and therefore may variably affect spermatogenesis.
Study design, size, duration: A retrospective analysis was performed on 125 men with leukaemia and 303 men with HL. Only those men who had sequential semen analyses (1 and 2) within a month were included. Volume, sperm concentration and motility were the selected parameters in samples 1 and 2. Time period was April 1980 to January 2021.
Participants/materials, setting, methods: We included all post-pubertal men diagnosed with 2 most common haematological malignancies (Hodgkin's lymphoma and leukaemia) in our database. Patient's demographics, cancer diagnosis and semen parameters were extracted from a secure electronic database and analysed using MS Excel. Cancer diagnoses were obtained from referral letters from oncologists. Differences between samples 1 and 2 were tested using Fisher's test, and odds ratios (OR) were calculated for the two malignancy groups.
Main results and the role of chance: We analysed 250 samples in 125 men with leukaemia and 606 samples in 303 men with HL. The mean intervals between the two semen samples were similar; 4.4 (1-30) and 3.8 (1-30) days. There were 95.7% of men <40 years in the HL group and 90.4% in the leukaemia group. There was no significant difference in the incidence of low volume (<1.5ml), sperm concentration or motility between samples 1 and 2 in both groups. Oligospermia was more frequently associated with leukaemia (OR 2.22, CI 95%, 1.44-3.43). Although the incidence of severe oligozoospermia was similar between the two cancer groups (OR 0.99, 95% CI 0.55 - 1.99), azoospermia was observed to have a greater association with leukaemia than HL (OR 3.22, 95% CI 1.57-6.63). There was also a greater association of asthenozoospermia with leukaemia compared to HL (OR 2.76, 95% CI 1.76-4.35). As there was consistency between samples 1 and 2 in both groups, odds ratio calculation for sample 2 revealed similar results as for sample 1.
Limitations, reasons for caution: As we selected men with at least two semen samples on two separate occasions, we had to exclude men with single samples which substantially reduced the number of participants. Types of leukaemia and the stage of disease in HL were not analysed.
Wider implications of the findings: Our findings are pertinent when counselling men about fertility preservation even in the absence of planned gonadotoxic treatment. Awareness about increased azoospermia incidence may help plan oncoTESE procedures. Our findings could form a basis for studies examining spermatogenesis pathways in haematological malignancies.
Trial registration number: not applicable

O-228 The SSRI antidepressant Sertraline inhibits CatSper calcium channels in human sperm

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Study question: Do Selective Serotonin Reuptake Inhibitor (SSRI) antidepressants affect the function of human sperm?
Summary answer: The SSRI-antidepressant Sertraline (e.g. Zoloft) inhibits the sperm-specific Ca^{2+} channel CatSper and affects human sperm function *in vitro*.
What is known already: In human sperm, CatSper translates changes of the chemical microenvironment into changes of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and swimming behavior. CatSper is promiscuously activated by oviductal ligands, but also by synthetic chemicals that might disturb the fertilization process. It is well known that SSRIs have off-target actions on Ca^{2+}, Na^+, and K^+ channels in somatic cells. Whether SSRIs affect the activity of CatSper is, however, unknown.
Study design, size, duration: We studied the action of the seven drugs belonging to the most commonly prescribed class of antidepressants, SSRIs, on resting $[Ca^{2+}]_i$ and Ca^{2+} influx via CatSper in human sperm. The SSRI Sertraline was selected for in-depth analysis of its action on steroid-, prostaglandin-, pH-, and voltage-activation of human CatSper. Moreover, the action of Sertraline on sperm acrosomal exocytosis and penetration into viscous media was evaluated.
Participants/materials, setting, methods: The activity of CatSper was investigated in sperm of healthy volunteers, using kinetic Ca^{2+} fluorimetry and patch-clamp recordings. Acrosomal exocytosis was investigated using *Pisum sativum* agglutinin (PSA) and image cytometry. Sperm penetration in viscous media was evaluated using the Kremer test.
Main results and the role of chance: Four SSRIs increased $[Ca^{2+}]_i$, two out of which also attenuated ligand-induced Ca^{2+} influx via CatSper. In contrast, Sertraline decreased $[Ca^{2+}]_i$ and almost completely suppressed ligand-induced Ca^{2+} influx via CatSper. Remarkably, the drug was about four-fold more potent to suppress prostaglandin- versus steroid-induced Ca^{2+} influx. Sertraline also suppressed alkaline- and voltage-activation of CatSper, indicating that the drug directly inhibits human CatSper. Finally, Sertraline suppressed ligand-induced acrosome reaction and sperm penetration into viscous media.
Limitations, reasons for caution: This is an *in vitro* study. Future studies have to assess the physiological relevance *in vivo*.
Wider implications of the findings: The off-target action of Sertraline on CatSper in human sperm might impair the fertilization process. In a research setting, Sertraline may be used to selectively inhibit prostaglandin-induced Ca^{2+} influx.
Trial registration number: CRU326

SELECTED ORAL COMMUNICATIONS

SESSION 80: NEW TWISTS IN OVARIANSTIMULATION - DO THEY WORK?

01 July 2021 Stream 3 14:00 - 15:15

O-229 Impact of letrozole co-treatment during ovarian stimulation with gonadotropins for in vitro fertilisation (IVF): a multicentre, randomised, double-blinded placebo-controlled trial

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Study question: Does reducing estradiol levels with letrozole co-treatment during ovarian stimulation with gonadotropins for IVF impact endocrinological and reproductive outcome markers in expected normal responders?
Summary answer: Letrozole co-treatment maintained follicular phase physiological serum estradiol levels, increased gonadotropin and androgen levels, and increased progesterone in the luteal phase.
What is known already: Ovarian stimulation for IVF causes supraphysiologic estradiol levels, which exert pituitary suppression reducing gonadotropin stimulation of the corpus luteum. Furthermore, stimulation may increase progesterone in the late follicular phase, reported to impair clinical outcomes, through a putative effect on endometrial maturation and embryo-endometrial asynchrony. Co-treatment with the highly selective aromatase inhibitor letrozole during ovarian stimulation has been shown to reduce estradiol levels and FSH consumption in poor responders, but conflicting data in relation to oocyte yield and implantation rates. The impact of letrozole co-treatment on hormonal changes and reproductive outcome after co-treatment in normal responders remains to be clarified.
Study design, size, duration: A multicentre double-blinded randomised placebo-controlled trial conducted in 4 fertility clinics at university hospitals in Denmark from August 2016 to November 2018. 159 women were randomised and 129 completed the study; 67 women in the letrozole group and 62 women in the placebo group. The study was conducted in accordance with the Helsinki Declaration and the ICH-Good-Clinical-Practice. Data collection and reporting followed the guidelines of CONSORT to achieve transparent reporting of trials.
Participants/materials, setting, methods: Women with expected normal ovarian reserve received an antagonist IVF protocol with fixed-dose FSH and fresh single embryo transfer. Co-treatment consisted of once-daily 5 mg letrozole or placebo from the start of stimulation until the day of triggering final oocyte maturation with human chorionic gonadotropin. Serum was collected on 7 visits from stimulation start to 8 days after oocyte retrieval. Clinical pregnancy was determined with a viable foetus by vaginal ultrasound at gestational week 7.
Main results and the role of chance: The proportion of patients with progesterone >1.5 ng/ml in the late follicular phase was similar in the letrozole versus placebo group with 6% versus 0%, respectively (OR 0, 95 % CI [0;1.6], P =.12). Mid-luteal progesterone levels >30 ng/ml were observed in 59% versus 31%, respectively, of subjects in the letrozole and placebo group (OR 3.3, 95% CI [1.4;7.1], P =.005). Letrozole treatment decreased estradiol levels by 69% (95 % CI [60%;75%], P <.0001) and increased luteinizing hormone (LH), testosterone, and androstenedione levels significantly in both the follicular and luteal phase. Follicle-stimulating hormone (FSH) concentration was elevated in the letrozole group at stimulation day 5 and at trigger day, and overall FSH consumption was diminished. The ongoing pregnancy rate did not differ between the letrozole and placebo group (31% versus 39% (risk-difference of 8%, 95% CI [-25%;11%], P =.55). Letrozole had no significant additional side effects apart from those frequently seen during ovarian stimulation, though a trend towards less nausea and vomiting was observed in the letrozole co-treated group versus the placebo group (28% versus 44% (risk-difference of 16%, 95% CI [-2%;33%], P =.11).
Limitations, reasons for caution: The diurnal variation of progesterone has been confirmed since this study was completed, hence the timing of the blood samples was not standardized . However, bias is unlikely due to the randomized design. The study was not powered to show an effect on ongoing pregnancy rates.
Wider implications of the findings: Letrozole co-treatment during ovarian stimulation with gonadotropins maintained serum estradiol at physiological levels, increased follicular phase levels of gonadotropins and androgens, and luteal progesterone levels. These data indicate that letrozole co-treatment may ameliorate the detrimental impacts of gonadotropin stimulation during IVF in normal responders.

Trial registration number: NCT02939898 and NCT02946684

O-230 RCT comparing Recombinant-hcg trigger with Dual-trigger (GnRH-agonist and recombinant-hcg) in improving clinical outcome in ICSI cycles in women with Diminished Ovarian Reserve

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Study question: Does use of Dual-trigger (GnRH-agonist with recombinant-hcg) improve the clinical outcome in women with diminished ovarian reserve as compared to Recombinant-hcg trigger?
Summary answer: Yes, the use of Dual-trigger (GnRH-agonist with recombinant-hcg) improve the clinical outcome in women with diminished ovarian reserve as compared to Recombinant-hcg trigger.
What is known already: The population of poor responders has grown exponentially over the years and their management of ovarian stimulation remains one of the most challenging aspects. In GnRH antagonist down-regulated IVF-ICSI cycles, dual triggering for the final oocyte maturation with GnRH-a and a reduced dose of hCG improves the rate of fertilization and clinical pregnancy in women with diminished ovarian reserve. Further more, the benefit of lowered cycle cancellation rate would also enable greater percentage of patients with diminished ovarian reserve to reach the final stage of their ART treatment, thereby enhancing their chances of achieving a successful pregnancy .
Study design, size, duration: This RCT included GnRH antagonist ICSI cycles from 2018-2019. 82 women with diminished ovarian reserve (AMH ≤ 1.1 ng/ml and AFC ≤5) were included. The primary outcome measured was the oocyte fertilization rate, implantation rate and clinical pregnancy rate per oocyte retrieval cycle. Secondary outcome measured was embryo transfer cancellation rate and abortion rate per oocyte retrieval cycle.
Participants/materials, setting, methods: 82 women with diminished ovarian reserve undergoing fresh embryo transfer were included and randomly divided in two groups - Group-A (hCG trigger/control group: n = 41); and Group-B (dual trigger/study group: n = 41). Both patient groups underwent controlled ovarian stimulation using antagonist. The final oocyte maturation was triggered either by recombinant hCG (Group-A) or by a combination of recombinant hCG and GnRH-agonist (Dual trigger) (Group-B).
Main results and the role of chance: The dual-trigger group had significantly higher fertilization rate (62.8 vs. 37.6%), higher clinical pregnancy rate (31.4% vs. 18.1%) as compared to the recombinant-hCG trigger group. In addition, the abortion rate(12.1% vs. 21.3%) and embryo transfer cancellation rate (8.3% vs. 16.1%) were both significantly lower in the dual trigger group. The baseline characteristics for the control and the study group were similar and there was no significant difference in the patient age, serum AMH level, and cause of infertility. The total r-FSH dose, duration of stimulation, endometrial thickness, and serum hormone profile on the day of trigger were also similar between the control and the study group. The main advantage of triggering with GnRH-a is that it induces a mid-cycle FSH surge which resembles the natural ovulatory cycle hormonal changes. Study shows that in GnRH antagonist ART cycles, dual triggering with GnRH-a and hCG could significantly improve the rate of fertilization and clinical pregnancy in diminished ovarian reserve women. Furthermore, the benefit of lowered cycle cancellation rate would also enable greater percentage of patients with diminished ovarian reserve to reach the final stage of their ART treatment thereby enhancing their chance of achieving a successful pregnancy as well as reducing their mental stress.
Limitations, reasons for caution: The main limitation of our study is the low patient number. Triggering with GnRH-a has become a significant part of contemporary ART practice, especially in high responders, oocytes donors and oncology patients. However, more RCTs are required in order to justify the use of GnRH-agonists in poor responders in ART cycles.
Wider implications of the findings: Results of our study concurred with other studies of dual triggering, calls for a possible paradigm shift in ovulation-triggering agent for GnRH-antagonist cycles. Diminished ovarian reserve patients are constituting a large part of clinical ART practice and for such patients, obtaining maximum mature oocytes and good embryos is vitally important.
Trial registration number: not applicable

O-231 In vitro maturation of human immature (GV) oocytes after controlled ovarian hormonal stimulation with recombinant AMH in the maturation medium

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Study question: Does the addition of recombinant AMH to the *in vitro* maturation (IVM) medium improve the maturation of GV oocytes after controlled ovarian hormonal stimulation?
Summary answer: Our results show that the addition of recombinant AMH to the *in vitro* maturation medium improves the maturation rate of GV oocytes.
What is known already: Anti-Müllerian hormone (AMH) is an important hormone involved in the process of sex differentiation during embryonic development. At the transition to the 21. century, more and more researchers have studied the role of AMH in ovarian function, especially its impact on folliculogenesis. AMH is becoming one of the main biomarkers of ovarian reserve and ovarian-specific disease, however, little is known about its effect on human oocyte maturation. Therefore, we matured immature GV (germinal vesicle) oocytes in IVM medium with recombinant AMH to assess its effect compared to the conventional IVM procedure with FSH and hCG.
Study design, size, duration: In this two-year prospective study, we compared the maturation rate of four groups of immature (GV) oocytes matured in maturation medium with added i) AMH (n=15), ii) AMH+FSH+hCG (n=44), iii) FSH+hCG (conventional; n=22), and iv) hormone-free maturation medium (control; n=15). Each oocyte was matured *in vitro* for a maximum of 28 hours and monitored by time-lapse microscopy to assess the time of GV breakdown (MI) and extrusion of the polar body (MII).
Participants/materials, setting, methods: Ninety-six GV oocytes of 46 patients (aged < 38 years, involved in the ICSI programme) after short antagonist protocol of controlled ovarian hormonal stimulation were included after written informed consent. IVM of oocytes was performed in the MediCult IVM System (LAG and IVM medium, Cooper Surgical, Denmark) with added hormones, and in a CO_2 incubator equipped with the PrimoVision time-lapse microscope (Vitrolife, Sweden).
Main results and the role of chance: IVM medium with added recombinant AMH gave the best result with all (100 %) oocytes matured *in vitro.* In conventional IVM medium with FSH and hCG, the oocyte maturation rate was poorer, with 68 % of oocytes matured *in vitro*. An even lower oocyte maturation rate (34 %) was observed in IVM medium with AMH, FSH and HCG, which might be explained by the antagonistic action of these hormones. In a group of control oocytes, 25 % of oocytes matured *in vitro*. The mean time to GV breakdown (MI stage) was 3.7 hours and to polar body release (MII stage) 20,5 hours. The time to MI stage was quite comparable in all groups of oocytes (3.5, 3.8 and 3.7 hours). There was a tendency for the polar body to be released later if AMH was added to the maturation medium (21.5 and 20.2 vs. 19.9 hours) but differences were not statistically significant, as revealed by Student's t-test. In the control group of oocytes, these times were prolonged (4.2 and 22.2 hours) due to slow spontaneous maturation. These preliminary results demonstrate that AMH could directly affect the oocyte maturation *in vitro*.
Limitations, reasons for caution: The limitation is the relatively small number of oocytes included; GV oocytes accounted for less than 10 % of all oocytes in the *in vitro* fertilisation (ICSI) programme. Moreover, the proportion of GV oocytes spontaneously matured to MI stage before the start of the experiment and were therefore not included.
Wider implications of the findings: Based on our data, we believe that AMH directly affects human oocyte maturation *in vitro*. Despite the common knowledge that AMH regulates the recruitment of growing ovarian follicles, it appears that the addition of AMH to the maturation medium can improve the human oocyte maturation *in vitro*.
Trial registration number: 0120-546/2018/6

O-232 Higher clinical pregnancy rate after oxytocin-receptor antagonist administration around the time of embryo transfer: A systematic review and meta-analysis of eleven RCTs

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Study question: Does the administration of an oxytocin-receptor antagonist around time of embryo transfer in IVF impact the likelihood to achieve a clinical pregnancy?
Summary answer: Administration of oxytocin-receptor antagonists around embryo transfer increases the likelihood of clinical pregnancy achievement.
What is known already: Uterine contractions occurring around time of embryo transfer have been described as one possible mechanism of failure of implantation of an embryo in the context of in-vitro fertilization (IVF). Hence the utilization of oxytocin-receptor antagonists was evaluated in randomized clinical trials (RCT) as a therapeutic approach. The compound Atosiban was studied by most RCTs (summarized in Huang *et al.* 2017). Recently further studies have become available which also investigated the novel agents Barusiban and Nolasiban. This systematic review collates the evidence of all drugs functioning as oxytocin-receptor antagonists which have been investigated in RCTs on IVF treatment so far.
Study design, size, duration: Multiple literature databases were searched for randomized controlled studies comparing the outcome of IVF cycles with administration of an oxytocin-receptor antagonist in the time period before, during or after embryo transfer versus placebo or *nil* in IVF patients. Meta-analyses were performed using standard procedures in the software program RevMan v.5.4. All analyses were done per randomized patient, wherever feasible.
Participants/materials, setting, methods: Eleven RCTs were identified and included in the meta-analysis. Seven utilized the agent Atosiban, one Barusiban and three Nolasiban. These drugs were administered either intravenously, subcutaneously or orally. The patient populations were heterogenous (fresh cycle, frozen-thawed cycle, endometriosis, implantation failure or general IVF-population) between trials. Only four studies reported live birth rates whereas all RCTs reported clinical pregnancy rate.
Main results and the role of chance: Administration of an oxytocin-receptor antagonist around embryo transfer increases the likelihood of live birth (relative risk: 1.1, 95% CI: 0.99-1.22, p=0.06, I^2=31%, four RCTs, n=2,510). Accordingly, the ongoing pregnancy rate is increased (relative risk: 1.14, 95% CI: 1.03-1.26, p=0.01, I^2=18%, four RCTs, n=2,510) as well as the clinical pregnancy rate (relative risk: 1.31, 95% CI: 1.13-1.51, p=0.0002, I^2=61%, eleven RCTs, n=3,611) by administration of an oxytocin-receptor antagonist. The risk to suffer a miscarriage, however, is not influenced by an oxytocin-receptor antagonist administration (relative risk: 0.90, 95% CI: 0.72-1.12, p=0.35, I^2=0%, seven RCTs, n=2,936). The risk of multiple pregnancy is not different between groups (relative risk: 1.05 95% CI: 0.81-1.36, p=0.73, I^2=5%, seven RCTs, n=3,014) as is the risk for an ectopic pregnancy (relative risk: 0.88 95% CI: 0.43-1.8, p=0.73, I^2=0%, four RCTs, n=2,714).
Limitations, reasons for caution: Methodological rigor is heterogenous between trials and some of the evidence is of poor quality. Evaluation of included studies is still ongoing and queries are pending. Additionally, there is heterogeneity between patient populations and definition of outcomes; only four RCTs report ongoing pregnancies and live births.
Wider implications of the findings: The administration of oxytocin-receptor antagonists around embryo transfer increases the pregnancy rate and may be a promising approach to enhance the likelihood to achieve a live birth per embryo transfer.
Trial registration number: n.a.

O-233 Micronized progesterone plus dydrogesterone versus micronized progesterone alone for luteal phase support in frozen-thawed cycles: a prospective cohort study

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Study question: Does the addition of oral dydrogesterone to vaginal progesterone as luteal phase support improve pregnancy outcomes during frozen embryo transfer (FET) cycles compared with vaginal progesterone alone?
Summary answer: Luteal phase support with oral dydrogesterone added to vaginal progesterone improves live birth rates and reduces miscarriage rates compared with vaginal progesterone alone.
What is known already: Progesterone is an important hormone that triggers secretory transformation of the endometrium to allow implantation of the embryo. During in vitro fertilization (IVF), exogenous progesterone is administered for luteal phase support. However, there is wide inter-individual variation in absorption of progesterone via the vaginal wall. Oral dydrogesterone is effective and well tolerated when used to provide luteal phase support after fresh embryo transfer. However, there are currently no data on the effectiveness of luteal phase support with the combination of dydrogesterone with vaginal micronized progesterone compared with vaginal micronized progesterone after FET.
Study design, size, duration: Prospective cohort study conducted at an academic infertility center in Vietnam from 26 June 2019 to 30 March 2020.
Participants/materials, setting, methods: We studied 1364 women undergoing IVF with FET. The luteal support regimen was either vaginal micronized progesterone 400 mg twice daily plus oral dydrogesterone 10 mg twice daily (second part of the study) or vaginal micronized progesterone 400 mg twice daily (first 4 months of the study). The primary endpoint was live birth after the first FET of the started cycle, with miscarriage <12 weeks as one of the secondary endpoints.
Main results and the role of chance: The vaginal progesterone + dydrogesterone group and vaginal progesterone groups included 732 and 632 participants, respectively. Live birth rates were 46.3% versus 41.3%, respectively (rate ratio [RR] 1.12, 95% confidence interval [CI] 0.99–1.27, p=0.06; multivariate analysis RR 1.30 (95% CI 1.01–1.68), p=0.042), with a statistically significant lower rate of miscarriage at <12 weeks (3.4% vs 6.6%; RR 0.51, 95% CI 0.32–0.83; p=0.009). Birth weight of both singletons (2971.0 ± 628.4 vs. 3118.8 ± 559.2 g; p=0.004) and twins (2175.5 ± 494.8 vs. 2494.2 ± 584.7; p=0.002) was significantly lower in the progesterone plus dydrogesterone versus progesterone group.
Limitations, reasons for caution: The study were the open-label design and the non-randomized nature of the sequential administration of study treatments. However, our systematic comparison of the two strategies was able to be performed much more rapidly than a conventional randomized controlled trial. In addition, the single ethnicity population limits external generalizability.
Wider implications of the findings: Oral dydrogesterone in addition to vaginal progesterone as luteal phase support in FET cycles can reduce the miscarriage rate and improve the live birth rate. Carefully planned prospective cohort studies with limited bias could be used as an alternative to randomized controlled clinical trials to inform clinical practice.
Trial registration number: NCT03998761

SELECTED ORAL COMMUNICATIONS

SESSION 81: IMPLANTATION AND EARLY PREGNANCY - EVENTS AND CONSEQUENCES

01 July 2021 Stream 4 14:00 - 15:15

O-234 Using serum metabolomics to identify biomarkers of viable early, intrauterine pregnancy: an untargeted 1H NMR-based approach

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Study question: Which metabolites are associated with a viable intrauterine pregnancy (VIUP) when compared to other early pregnancy outcomes (failed intrauterine and ectopic pregnancies)?
Summary answer: Serum levels of four metabolites (phenylalanine, alanine, glutamate and glutamine) were significantly altered in VIUPs compared to other early pregnancy outcomes.
What is known already: Around 10% of all intrauterine pregnancies are lost in the first trimester. A further 1-2% of pregnancies are located outside the endometrial cavity; these ectopic pregnancies are the leading cause of maternal mortality in the first trimester of gestation. Early miscarriages may also cause significant morbidity when bleeding or infection occurs. The symptoms of miscarriages and ectopic pregnancy are often similar (pain and bleeding), however, such symptoms are also common in VIUPs. To date, no biomarkers have been identified to differentiate VIUPs from non-viable and ectopic pregnancies.
Study design, size, duration: This is a prospective cohort study that included 332 pregnant women at less than ten weeks of gestation, who attended the early pregnancy assessment unit (EPAU) at Liverpool Women's Hospital with pain and/or bleeding.
Participants/materials, setting, methods: Blood samples were collected from the 332 pregnant women prior to final clinical diagnosis of pregnancy outcome. Serum samples were subjected to NMR metabolomics profiling (14 spectra that did not meet the recommended minimum reporting standards were removed from subsequent analysis). 1D ^{1}H-NMR spectra were acquired at 37 °C on a 700 MHz spectrometer. Relative metabolite abundances underwent statistical analysis using MetaboAnalyst 5.0 (*p*-value FDR adjusted).
Main results and the role of chance: Final pregnancy outcomes were as follows: one hydatidiform mole (0.3%), 48 ectopic pregnancies (14.4%), three pregnancies of unknown location (PULs, 0.9%), 78 failed pregnancies of unknown location (FPULs, 23.4%), 47 miscarriages (14.1%), two vanishing twin pregnancies (0.6%) and 153 VIUPs (45.8%). Due to small sample numbers, the hydatidiform mole, PULs and vanishing twin pregnancies were excluded from further analysis. To compare VIUPs to other pregnancy outcomes, ectopic pregnancies, FPULs and miscarriages were grouped together. Univariate analysis of serum metabolite concentrations identified four metabolites (phenylalanine, alanine, glutamate and glutamine) as significantly different in VIUPs compared to other pregnancy outcomes. Multivariate partial least squared discriminant analysis provided only weak correlation between the serum metabolome and pregnancy outcome. In summary, we have identified differences in the metabolome of women with VIUPs compared to other common pregnancy outcomes, which may provide diagnostic utility.
Limitations, reasons for caution: In this study, women with VIUPs presented with pain and/or bleeding. The presence of symptoms may influence the metabolome of this group versus VIUPs without symptoms, thus limiting the translation of our findings. Furthermore, environmental factors were not controlled (e.g. fasting status), making it likely that cohort heterogeneity was enhanced.
Wider implications of the findings: This study identifies a metabolite profile associated with VIUPs. These findings may be useful in the development of a diagnostic test to confirm VIUPs and thus exclude potentially life-threatening pregnancy outcomes. Such a test would be invaluable in clinical emergencies.
Trial registration number: NA

O-235 ERICA (Embryo Ranking Intelligent Classification Assistant) AI predicts miscarriage in poorly ranked embryos from one static, non-invasive embryo image assessment

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Study question: Does ERICA's prognosis ranking based on ploidy, predict early miscarriage following positive biochemical pregnancy test?
Summary answer: The lower ERICA grades embryos, the higher the likelihood of early miscarriage, irrespective of age group.
What is known already: The vast majority of early miscarriages are due to aneuploidy, but preimplantation genetic testing for aneuploidy (PGTA) is potentially invasive, expensive, time-consuming and usually necessitates cryopreservation. Current methods for embryo selection based on morphology and morphokinetics are poorly correlated with ploidy. ERICA is a deep-learning non-invasive tool for embryo ranking, trained to identify ploidy, and has previously been shown to be similar or better than experienced embryologists in assessing implantation potential. AI-based tools capable of embryo ranking and assessment could help save laboratory time and costs, avoiding risk to embryos from invasive techniques.
Study design, size, duration: Retrospective analysis of 599 blastocysts transferred over 12 months in which ERICA was used to assist embryologists during the embryo selection process. ERICA's prognosis based on ploidy potential is presented as groups labelled as "optimal", "good", "fair", or "poor". Embryo transfers (ET) reaching biochemical pregnancy (beta-hCG ≥ 20iu) were considered for the study. Early pregnancy loss (EPL) was defined as a biochemical pregnancy failing to develop a gestational sac and/or failure to show heartbeat (FHR).
Participants/materials, setting, methods: ETs resulting in biochemical pregnancies at two IVF clinics were followed-up to FHR till 8 weeks gestation. EPLs were divided into groups according to the presence or absence of a pregnancy sac. ERICA's suggested prognosis during the embryo selection process was tested against pregnancy outcomes. Further analysis of pregnancy outcomes and their relation to ERICA's labels was also performed based on age groups. Z-test for two proportions was used to assess statistical significance.
Main results and the role of chance: 506 ETs were performed for 599 embryos (mean 1.2 embryos), from which 285 resulted in positive pregnancy tests (56.3%). Thirty-one (10.9%) EPLs happened before the identification of a gestational sac (GS). Ten pregnancies failed to develop FHR after initial GS identification (3.9%), for an overall EPL of 14.4%. The average age in this group was 35.4 years. When evaluated using ERICA's labels "optimal", "good", "fair, and "poor", chances of miscarriage before GS were 8.9% (8/89); 14.1% (11/78); 18.5% (5/27); and 18.7% (9/48) respectively, where denominator represents total number within a label (i.e. EPL/n). When including all EPLs, chances of miscarriage according to the same labels were 11.2%; 17.9%; 22.2%; and 22.9% respectively.

ERICA's performance to anticipate the risk of EPL showed statistical significance when the optimal label was compared against all other labels (Z -1.786, $p < 0.05$), and against the poor prognosis label (Z=-1.653, $p<0.05$). After stratifying the dataset according to age groups, increasing miscarriage rates were maintained as ERICA's prognosis for an embryo worsened, regardless of age groups. The most notable performance was for ≤35-year-olds, where embryos ranked as optimal had an EPL rate of 14.3% in contrast to lowest ranked embryos having a 33.3% EPL rate.
Limitations, reasons for caution: The retrospective nature of this study along with its sample-size might limit the reach of our conclusions, in particular for older patients. The results we present must still be confirmed prospectively, and on a larger dataset.
Wider implications of the findings: Most EPLs are attributed to genetic factors, hence ERICA's training for embryo ranking was based on ploidy. We conclude that ERICA's AI is able to identify embryos at a higher risk of EPL non-invasively. Cytogenetic studies from products of miscarriage would help to confirm the hypothesis.
Trial registration number: Not applicable

O-236 The clinical and morphokinetic factors indicating a risk of pregnancy loss after a euploid embryo transfer

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Study question: Are there any clinical and morphokinetic factors which may affect the pregnancy outcome after a euploid embryo transfer?
Summary answer: Body mass index (BMI), endometriosis, the history of recurrent pregnancy losses and the number of previous frozen-thawed unsuccessful embryo transfer (FET) cycles impact pregnancy outcomes.
What is known already: Preimplantation genetic testing for aneuploidy (PGT-A) is largely used for various indications to detect chromosomal abnormalities in assisted reproductive technologies (ART). The most common reason for the first trimester pregnancy losses is chromosomal abnormalities. However, the factors that cause pregnancy loss after a euploid embryo transfer are not fully understood.
Study design, size, duration: The pregnancy results of all single euploid embryos tested with next generation sequencing (NGS) in Istanbul Memorial Hospital between January 2017 and March 2020 were evaluated in this single center retrospective cohort study. The cases that resulted in pregnancy below the age of 43 were analyzed according to outcomes; biochemical pregnancy loss (Group 1), clinical pregnancy loss (Group 2) and live birth (Group 3).
Participants/materials, setting, methods: The transfer of 2041 single euploid embryos resulted in 1492 pregnancies. The clinical and morphokinetic parameters observed using time lapse imaging (TLI) were compared among the three groups.
Main results and the role of chance: The overall pregnancy rate was 73.1%, the rates of biochemical pregnancy losses and clinical losses were 9.7% and 11.4% respectively. The live birth rate was 58.5%. The indications for PGT-A were as follows; recurrent pregnancy losses (RPL) (14.9%), recurrent implantation failure (RIF) (11.7%), advanced maternal age (AMA) (28.6%), a history of abnormal fetal karyotype or single gene defects (12.1%). In 32.6% cases PGT-A was performed to reduce time to pregnancy.

There were no differences in terms of female age, AMH, the diagnosis or the duration of infertility, the mean numbers of oocytes retrieved, mature and fertilized oocytes. However, BMI values, the presence of severe endometriosis, including adenomyosis, the history of recurrent pregnancy losses and the number of previous unsuccessful FET cycles were significantly higher in Groups 1 and 2.

When pregnancy losses were evaluated according to PGT indications, patients with a history of RPL had a significantly higher pregnancy loss rate (27.8%) compared to the other groups: AMA (19.6%), RIF (19.4%), genetic factors (21.6%) and cases where PGT was performed to reduce time to pregnancy (16.4%) ($p<0.05$).

When morphokinetic parameters were evaluated, they were found to be not significantly different in the three groups ($p>0.05$).
Limitations, reasons for caution: The retrospective nature of the data is the major limitation of the study. On the other hand, the strength of the study is the large number of PGT-A tested embryos from a single center which used the same laboratory conditions.
Wider implications of the findings: PGT-A is widely used to avoid pregnancy losses. However, BMI values, the presence of severe endometriosis, including adenomyosis, the history of recurrent pregnancy losses and the number of previous unsuccessful FET cycles should be taken into consideration during counselling and/or treatment.
Trial registration number: not applicable

O-237 A non-invasive approach for aneuploidy analysis in clinical miscarriages

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Study question: Is maternal cell-free DNA (cfDNA) testing a feasible alternative to the analysis of the product of conception (POC) in clinical miscarriages?
Summary answer: This study demonstrates that genome-wide cfDNA testing in the maternal bloodstream constitutes a reliable tool to analyse chromosome aneuploidies in clinical miscarriages.
What is known already: It is well established that 50-70% of clinical miscarriages are caused by numerical chromosomal anomalies (aneuploidies), mostly trisomies. To date, conventional cytogenetic and advanced molecular techniques are used for the analysis of POC to identify the genetic cause of miscarriage, providing valuable information for genetic counselling. However, both approaches are based in the direct analysis of the abortive tissue, which entails several limitations due to the risk of culture failure and/or maternal cell contamination. To solve these drawbacks, maternal cfDNA testing emerges as a promising alternative due to the accumulated evidence.
Study design, size, duration: This was a retrospective study conducted in a reference genetic laboratory from January to December 2020. Before carrying out the foetal tissues collection that precludes the POC analysis, a blood sample was drawn to evaluate possible aneuploidies by cfDNA testing. Using NGS+STR POC results as the gold standard, results derived from both studies were compared to assess the percentage of concordance and the cases of non-informativeness (foetal fraction (FF) <2%), false positives, and false negatives.
Participants/materials, setting, methods: A total of 12 cases were included in the study. cfDNA testing in the mother's blood was performed by using Illumina's technology platform. Genetic testing for POC was done using an NGS technology (Thermo Fisher Scientific, USA) for 24 chromosome aneuploidy screening. Short-tandem repeat (STR) analysis allowed us to detect or rule out maternal cell contamination (MCC) and some types of polyploidies.
Main results and the role of chance: The non-informative rate for both analysis techniques was 9.1% (1 out of 12 cases: 1 low FF for cfDNA testing and maternal cell contamination for POC analysis). The median cfDNA FF was 9.0%. Using the molecular POC analysis as gold standard, the concordance rate between both studies was 90.0% (9 out of 10 cases;1 monosomy X, 1 trisomy (T) 21, 1 T22, 1 T11 and 5 patients with no alteration detected). No mosaics or structural rearrangements were identified by either of the two analysis techniques. The only discordant result was a case in which cytogenetics of POCs identified a triploidy. This discordancy is expected since triploidies are outside the scope of cfDNA testing. Also, foetal sex was correctly assigned in all informative cases. The sensibility and specificity of the study were estimated at 80.0 (4/5) and 100.0% (6/6), respectively. Statistics analysis suggested that no significant difference was found between both techniques regarding the aneuploidy detection ability ($P=0.5$). These promising results indicate that genome-wide cfDNA-based screening provides a non-invasive approach for determining whether foetal aneuploidy could explain the loss in patients experiencing early o recurrent pregnancy loss (RPL).
Limitations, reasons for caution: The sample size prevents drawing more significant conclusions regarding the diagnosis power similarity between both testing techniques. Therefore, a larger cohort will be essential to improve confirm the cfDNA testing performance. Current cfDNA testing technology fails in polyploidy identification, which is a potential cause of pregnancy loss.
Wider implications of the findings: CfDNA testing could be an alternative to POC analysis in clinical miscarriage. If optimized, cfDNA testing could be used contingently with the molecular POC analysis in cases where maternal cell contamination is present. As a result, the overall success rate in the POC program could be substantially improved.
Trial registration number: NA

O-238 Preterm birth after recurrent pregnancy loss: A systematic review and meta-analysis

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Study question: What is the impact of recurrent pregnancy loss on the risk of preterm birth?

Summary answer: Women with RPL were found to be at increased odds of having preterm deliveries (<37 weeks gestation) in their subsequent live pregnancies.

What is known already: Recurrent pregnancy loss (RPL) occurs in up to 5% of all women with miscarriages. The emotional, physical, and financial burden associated with RPL is unequivocal, and over the years, much research has gone into the management of RPL. However, relatively little is known about the perinatal outcomes following RPL. Past research in the area reports conflicting data on the association between RPL and preterm birth (PTB) in a subsequent pregnancy.

Study design, size, duration: A systematic search was performed across the-PubMed, EMBASE and Google Scholar databases for relevant studies published up until October 2020. Observational cohort and case-control studies comparing the risk of preterm birth (PTB) among women with and without a history of RPL were included. Effect estimates were pooled using a DerSimonian and Laird random-effects meta-analysis model. Study appraisal was performed using the Newcastle-Ottawa scale.

Participants/materials, setting, methods: We included studies where the study population consisted of women with a history of RPL (defined as 2 or more pregnancy losses), where the comparator group consisted of women without a history of RPL, and where the outcomes assessed included PTB (defined as birth prior to 37 completed weeks gestation). Two reviewers independently extracted data in duplicate. Publication date, population, exposure and outcome data were extracted.

Main results and the role of chance: A total of 12 retrospective observational studies met inclusion criteria, and were included in the systematic review and meta-analysis (N = 37,046 women with a history of RPL). Incidence of PTB among the RPL groups ranged from 5.8% to 19.6%, and from 1.5-14.0% in the non-RPL groups. A pooled OR of 1.59 with 95% CI 1.40-1.80 was observed in our random-effects meta-analysis with an I^2 of 84%. Subgroup analyses were completed for the pooled risk of only 2 RPL (pooled odds ratio [OR] 1.35; 95% CI 1.08-1.69; I^2=84.7%); ≥2 RPL (pooled OR 1.42; 95% CI 0.91-2.22; I^2=68.9%); and ≥3 RPL (pooled OR 1.86; 95% CI 1.51-2.29; I^2=79.5%).

Limitations, reasons for caution: Inconsistent adjustment for confounders and significant between-study heterogeneity were noted in this study.

Wider implications of the findings: Despite significant heterogeneity among studies, we found that women with a history of RPL had significantly higher odds of delivering preterm infants in their subsequent pregnancies.

Trial registration number: CRD 224763

human reproduction

ESHRE 2021 / Poster Viewing

POSTER VIEWING
ANDROLOGY

P-001 Effect of mild α-chymotrypsin treatment of highly viscous semen samples on fertilization rates

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Study question: The main objective of this study was to examine the influence of mild α-chymotrypsin treatment of highly viscous semen samples on the fertilisation rate after artificial reproductive treatment (ART).
Summary answer: The use of mild α-chymotrypsin treatment of semen samples in case of hyperviscosity does not appear to impact negatively on the fertilization rates after ART.
What is known already: Highly viscous semen reduces sperm motility significantly and can contribute to infertility. When processing semen samples, few techniques exist to induce liquefaction in case of seminal hyperviscosity such as different washing steps and mechanical treatment. The use of α-chymotrypsin seems controversial due to possible negative effects on fertilisation rates after IVF.
Study design, size, duration: All patients were recruited at the Department of Gynecological Endocrinology and Reproductive Medicine, University Hospital of Bonn, Germany from July first 2018 till June first 2019. Anonymized data on age, number of previous pregnancies and deliveries were retrospectively collected. The study group consisted of a cohort of 52 couples meeting the inclusion criteria of highly viscous semen and were compared to a cohort control group of 88 couples.
Participants/materials, setting, methods: The fertilization rate of 52 ART cycles was examined following IVF using a low dose of α-chymotrypsin to induce liquefaction of highly viscous semen and was compared to a control group of 88 ART cycles. Data was analyzed using SPSS version 25. A Mann Whitney U test was used to compare continuous parameters between groups.
Main results and the role of chance: The study group consisted of a cohort of 52 couples meeting the inclusion criteria of highly viscous semen and were compared to a cohort control group of 88 couples. The Fertilization rate between the two groups was not significant ($p<0.146$, Mann-Whitney U test), with a rate of 57.95 in the study group compared to 57.53 in the control group. Our analysis showed no significant differences in male and female age, male abstinence period, semen volume, sperm concentration, total sperm count, and total progressive sperm count between the two groups. We observed a significant difference [$p=0.025$] in the progressive motility and a borderline significance in the total progressive sperm count [$p=0.052$] between α-chymotrypsin treated samples and the non α-chymotrypsin treated samples. Analyzing the clinical and biochemical pregnancy parameters of the study group (n=41) and the control group (n=66) per fresh embryo transfer procedures (n=107) we found no significant differences. Freeze all cycles were excluded from the study. No significant differences concerning previous pregnancies and childbirth were detected.
Limitations, reasons for caution: The study is a pilot study as the majority of the studies using α-chymotrypsin was conducted up to three decades ago, comparison of data must be interpreted in the light of the fact that since then, IVF has seen a rapid evolution of technology and culture techniques.
Wider implications of the findings: The use of mild α-chymotrypsin treatment of semen samples in case of hyperviscosity does not appear to impact negatively on the fertilization rates after ART and could be regarded as an additional method to induce liquefaction of highly viscous semen samples in IVF...
Trial registration number: not applicable

P-002 Prediction model for salvage microdissection testicular sperm extraction in patients with failed conventional testicular sperm extraction

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Study question: Is there any intra-surgical parameter able to predict the outcome of salvage microdissection testicular sperm extraction (mTESE) in patients with previous failed TESE?
Summary answer: Among all the variables under consideration, only the seminiferous tubules (ST) caliber pattern found at high magnification was able to significantly predict the mTESE outcome...
What is known already: Several studies have demonstrated that no clinical or hormonal parameters are able to predict the outcome of a salvage mTESE performed in patients with previous sperm retrieval failure (SRF). It has been previously demonstrated that a prediction model with the combination of two intra-surgical parameters such as the STs caliber, defined as dilated tubule (DT), slightly dilated tubules (SDT) and not dilated tubules (NDT), and testis histology had an excellent discrimination ability (AUC 0.93) to distinguish between cases with and without the outcome, but such prediction model has not been tested in patients undergoing salvage mTESE.
Study design, size, duration: A prediction model was built on a dataset of 63 patients, 29-50 years old, undergoing unilateral (15) or bilateral (48) salvage mTESE after failed TESE from 2015 through 2019, with a resulting N=111 testes under consideration. Two models were compared, one with STs and histology as covariates, the other with STs alone: the second model was chosen due to better discrimination...
Participants/materials, setting, methods: we assessed internal validity with a bootstrapping procedure for a realistic estimate of the performance of the prediction model in similar future patients with NOA undergoing salvage mTESE. We repeated the entire modeling process in 259 samples drawn with replacement from the original sample, and determined the performances (AUC, sensitivity, specificity) of the selected prediction model. Calibration (correspondence between the predicted and observed probabilities) was visually assessed by inspecting the calibration belt...
Main results and the role of chance: Sperm retrieval was successful in 24 out of 63 patients (38%): age, testis volume and hormonal parameters did not vary among patients with successful sperm retrieval (SSR) or SRF. The prevalent histological pattern was Sertoli cell only syndrome (69.6%), while hypospermatogenesis, maturation arrest and hyalinosis were found in 4.5%, 23% and 1.8% of cases. The STs pattern was heterogeneous, with DTs being found only in 23.4% of testes. Sperm were found in 69% of DTs, 29% of SDTs, and 5% of

NDTs. The prediction model correctly classified 82.88% of patients and explained the 26.5% variability of the outcome. The STs pattern significantly predicted the mTESE outcome with a sensitivity of 62% and a specificity of 90.2%, PPV 69.2%, NPV 87%. Both SDT (OR 0.105, 95% CI 0.034-0.317, p<0.0001) and NDT (OR 0.024, 95% CI 0.004-0.128, p<0.0001) were negatively associated with the chance of retrieving sperm, the resulting prediction equation being Log (SSR)= 0.81 – 2.2 SDT – 3.7 NDT. The model had a clearly useful discrimination (AUC 0.813). The optimism corrected AUC was 0.7977, and the model was well calibrated (p=1.00) with both the 80% and 95% calibration belts encompassing the bisector over the whole range of the predicted probabilities

Limitations, reasons for caution: The STs caliber pattern was subjectively evaluated at high magnification (24-36x) by comparing the individual ST appearance with the surrounding ones, however such evaluation was performed by the same experienced urologist with more than 1000 mTESE procedures performed to date.

Wider implications of the findings: No clinical data but the STs appearance at high magnification could discriminate between patients with and without chances of SSR. These results reinforce the evidence supporting the superiority of mTESE compared to conventional TESE in retrieving sperm, particularly in lower prognosis patients with NOA such as those with previous SR.

Trial registration number: Not applicable

P-003 Transposon insertion profiling by sequencing (TIPseq) identifies novel LINE-1 insertions in human sperm

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Study question: Do human sperm contain novel LINE-1 insertions and are they affected by paternal age?

Summary answer: Human sperm contain novel LINE-1 insertions. Their location or number are not affected by paternal age.

What is known already: LINE-1 comprises 17% of the human genome and some LINE-1s are the only autonomous retrotransposons in humans. Retrotransposons influence genomic instability and/or regulation if new retrotransposition events disrupt coding or regulatory regions in the host genome. Demethylation during germ cell development de-represses retrotransposons. Advanced paternal age is associated with genomic instability. Previously we showed that sperm LINE-1 copy number decreases with paternal age. We hypothesize that human sperm exhibit de novo retrotransposition and that sperm from older men contain increased novel LINE-1 insertions.

Study design, size, duration: Cross-sectional case–control study with semen samples collected between February to July 2020.

Participants/materials, setting, methods: Normospermic sperm samples (n=10; 5 <35 years old and 5 ≥45 years old) obtained from consenting men undergoing IVF at NYU Fertility Center were submitted to a novel method, single cell Transposon Insertion Profiling by Sequencing (scTIPseq) to identify and map LINE-1 insertions in human sperm. TIPseqHunter, a custom bioinformatics pipeline, compared the architecture of sperm LINE-1 to known LINE-1 insertions from the European database of human specific LINE-1 (L1Hs) retrotransposon insertions in humans (euL1db).

Main results and the role of chance: TIPseq identified 17 novel insertions in sperm, 8 from older (≥ 45 years) and 9 in younger men (<35 years). New insertions were mainly intergenic or intronic, including *AC007402* (2/10), *TMEM163* (2/7), *CTTNBP2NL* (3/5), *AC107023* (3/3), *TMC2* (2/19), *MacroD2* (2/6), *RAB3C* (3/4), *LINC02664* (1/1), *AC079052* (2/3) and *AC017091* (4/4). One novel insertion (<35 years old) hits a known regulatory element. Only one sample (≥ 45 years old) did not exhibit any new insertion. The location or number of novel insertions did not differ by paternal age.

Limitations, reasons for caution: The small sample-size and use of normospermic specimens limit interpretation of paternal age effect on LINE-1. Besides, the novel insertions could be polymorphic sites that have low allele frequency and thus have not yet been described.

Wider implications of the findings: This study for the first time reports novel LINE-1 insertions in human sperm, demonstrating that scTIPseq method is a feasible technique, and identifying new contributions to genetic diversity in the human germ line. Further studies are needed to evaluate the impact of these insertions on sperm function.

Trial registration number: Not applicable

P-004 Effect of varicocelectomy on sperm DNA fragmentation rates in infertile men with clinical varicocele: a systematic review and meta-analysis

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Study question: Does varicocelectomy improve sperm DNA quality in men with infertility and clinically detected varicoceles?

Summary answer: Varicocelectomy reduces sperm DNA fragmentation (SDF) rates in infertile men with clinical varicocele.

What is known already: Varicocele has been linked to male infertility through various non-mutually exclusive mechanisms, including an increase in reactive oxygen species (ROS) production that may lead to sperm DNA damage. Damage to sperm DNA may result in longer time-to-pregnancy, unexplained infertility, recurrent pregnancy loss, and failed intrauterine insemination or in vitro fertilization/intracytoplasmic sperm injection. Therefore, interventions aimed at decreasing SDF rates, including varicocele repair, have been explored to improve fertility and pregnancy outcomes potentially, either by natural conception or using medically assisted reproduction.

Study design, size, duration: Systematic review and meta-analysis

Participants/materials, setting, methods: We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Our systematic search included PubMed/Medline, EMBASE, Scielo, and Google Scholar to identify all relevant studies written in English and published from inception until October 2020. Inclusion criteria were studies comparing SDF rates before and after varicocelectomy in infertile men with clinical varicocele. Articles were included if the following SDF assays were utilized: SCSA, TUNEL, SCD test, or alkaline Comet.

Main results and the role of chance: Thirteen studies fulfilled the inclusion criteria and were selected for the analysis. The estimated weighted mean difference of SDF rates after varicocelectomy was -6.58% (13 studies, 95% CI -8.33%, -4.84%; I^2=90% p<0.0001). Subgroup analysis revealed a significant decrease in SDF rates using SCSA (eight studies, WMD -6.80%, 95% CI -9.31%, -4.28%; I^2=89%, p<0.0001), and TUNEL (three studies, WMD -4.86%, 95% CI -7.38%, -2.34%; I^2=89%, p<0.0001). The test for subgroup difference revealed that pooled results were conservative using the above SDF assays. Comet and SCD tests were used in only one study each; thus, a meta-analysis was not applicable. The studies were further categorized by the surgical technique (microsurgical versus non-microsurgical). This subgroup analysis showed a significant decrease in SDF rates using microsurgical technique (10 studies, WMD -6.70%, 95% CI -9.04%, -4.37%; I2=91%, p<0.0001). After varicocelectomy, SDF rates were also decreased when non-microsurgical approaches were used, albeit the effect was not statistically significant (2 studies, WMD -6.84%, 95% CI -10.05%, 1.38%; I2=86%) (Figure 3). The heterogeneity was not materially affected by performing analyses by the above subgroups, suggesting that the SDF assay and surgical technique do not explain the inconsistency in the treatment effect across primary studies.

Limitations, reasons for caution: There were no randomized controlled trials comparing varicocelectomy to placebo for alleviating SDF levels. Heterogeneity was high, which may be explained by the low number of included studies. Pregnancy data are not available in most studies, thus the impact of reduced SDF after varicocelectomy on pregnancy rates unclear.

Wider implications of the findings: Our study indicates a positive association between varicocelectomy and reduced postoperative SDF rates in men with clinical varicocele and infertility, independentetly of the assays used to

measure SDF. These findings may help counsel and manage infertile men with varicocele and high SDF levels.
Trial registration number: not applicable

P-005 Magnetic-activated cell sorting in couples undergoing preimplantation genetic testing for aneuploidies (PGT-A) using autologous oocytes shows slightly lower aneuploidy rates compared to standard semen processing

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Study question: Does the selection of non-apoptotic sperm via magnetic-activated cell sorting (MACS) reduce the aneuploidy rate of embryos from couples undergoing ICSI cycles with PGT-A using the patients' own oocytes?
Summary answer: It does. The aneuploidy rate in the MACS group was 4.34% lower than the one obtained using semen samples processed according to standard clinical practice.
What is known already: MACS is a successful tool in eliminating proapoptotic sperm from a semen sample. However, the true effect of this technique on reproductive outcomes and the quality of the resulting embryos are a matter of controversy. Some studies report that its use improves the percentage of good quality blastocysts in women older than 30 years old compared to standard ICSI. Randomized clinical trials that compare MACS to a control sample consider parameters of embryo quality such as morphology at day 3 or day 5, symmetry of the blastomeres, blastocysts' stage of expansion, but they do not consider embryo ploidy.
Study design, size, duration: Retrospective, multicentre, observational cohort study. 14,145 patients and 18,710 cycles were evaluated in the reference group. In the MACS group, 615 patients and 974 cycles were considered. Data were exported from cycles performed in Spanish IVIRMA clinics between January 2008 and February 2020.
Participants/materials, setting, methods: Unselected males in couples undergoing PGT-A cycles, then subdivided into male factor (MF) - total progressive motile sperm count lower than 5 million - and non-male factor (NMF) infertility. Statistical analysis performed using R v.4.0.0. Means were calculated and compared using two-tailed paired t-test, while proportions were compared using Fisher's exact test and the chi-squared test and the appropriate correction for multiple comparisons. The aneuploidy rates for each group were compared using Fisher's exact test.
Main results and the role of chance: In the control group 73,228 biopsied embryos, from which 71,439 were informative in the PGT-A. In the MACS group 3,919 biopsied embryos, from which 3,843 were informative. The aneuploidy rate, computed per informative embryo, was 68.87% (68.40%, 69.34%) in the reference group and 64.53% (62.43%, 66.64%) in the MACS group. Both comparisons were statistically significant (p-value <0.00001). According to these results, an embryo in the PGT-A programme using non-apoptotic sperm selected through MACS and autologous oocytes had a 5% less chance of being aneuploid than those embryos fertilised with standardly selected sperm (relative risk of 0.95 (0.91-0.98) p=0.006769). Embryos conceived from NMF patients whose semen had been processed using MACS had a 4.27% lower aneuploidy rate than the reference (65.52% (63.16%, 67.88%) vs 69.79% (69.20%, 70.37%) respectively). This difference was statistically significant. Those embryos conceived using semen from patients with MF using MACS also showed a lower aneuploidy rate than the reference with MF (0.28% (55.48%, 65.08%) vs (64.94% (63.35%, 66.23%) respectively), although this difference was not statistically significant. Thus, the decrease in aneuploidy rate observed when comparing MACS and reference groups undergoing PGT-A cycles using autologous oocytes remained approximately the same in both MF and NMF semen samples.
Limitations, reasons for caution: The retrospective nature of the study subjects the data to biases or inaccuracies in their annotation in the clinics' informatic platform from which they were exported. However, the statistical analysis aimed at controlling these biases as much as possible.
Wider implications of the findings: The vast amount of data compiled for this study confirms that the selection of non-apoptotic sperm through MACS slightly decreases the aneuploidy rate of embryos compared to semen samples processed according to the clinics' standards. This would be interesting for patients who are considering undergoing PGT-A cycles in the future.
Trial registration number: Not applicable

P-006 Simultaneous determination of bisphenol A and S in the samples of human seminal fluid

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Study question: Can we quantitatively determine concentrations of endocrine disruptors namely bisphenol A and S in seminal fluid?
Summary answer: We developed selective analytical method to simultaneously screen for the presence of bisphenol A (BPA) and S (BPS).
What is known already: The male reproductive system involves processes, which may be influenced by the disruption of the endocrine system by chemicals called endocrine disruptors (EDs). There is a growing evidence that EDs such as bisphenol A and S may be responsible for the decline in male reproductive health. To date, the claimed adverse effects on male fertility are largely based on the results from studies assessing the relationship between urinary BPA and BPS concentration and semen parameters. The best evidence of an adverse effect of BPA and BPS directly on spermatozoa could be provided by measuring bisphenols concentration directly in seminal fluid.
Study design, size, duration: To selectively and quantitatively analyzed bisphenols in any biological matrix advanced analytical tools and selective sample preparation protocols must be employed. In this study we developed targeted analytical method based on liquid chromatography tandem mass (LC-MS/MS) detection to measure bisphenol A and S in seminal fluid samples obtained from IVF clinic. A total of 140 samples were analysed.
Participants/materials, setting, methods: BPA and BPS was extracted from 140 seminal fluid samples using solvent extraction followed by preconcentration step. Samples were analyzed on Agilent 6495 Triple Quadrupole (Agilent Technologies, Santa Clara, CA) operating in the ESI-negative mode. Two MS/MS transitions were used for quantitative LC-MS/MS analyses. Chromatographic separation was achieved on Waters™ ACQUITY™ UPLC™BEH C18 (100 × 2.1 mm, 1.7 µm) column using gradient elution with a mixture of 0.1mM ammonium fluoride and methanol as mobile phases.
Main results and the role of chance: We developed selective sample preparation method for detection of BPA and BPS in seminal fluid followed by LC-MS/MS detection. The method validation was performed based on FDA guidelines. Validation criteria included limit of detection (LOD), limit of quantitation (LOQ), accuracy and precision. Due to the lack of the certified reference material the validation criteria of the method were assessed in pool of spiked seminal samples. The accuracy of the LC-MS/MS method was evaluated as a percent recovery of the amount of target analyte added into the sample. Recovery rates were above 80 % for both analytes. LOD was 0.04 ng/mL for BPA and 0.01 ng/mL for BPS. LOQ was 0.14 ng/mL and 0.02 ng/mL for BPS. Measured BPA concentration ranged from 0.04 ng/mL to 1.62 ng/mL. For BPS, the concentration ranged from 0.01 ng/mL to 0.47 ng/mL. BPA and BPS were detected in 64 % and 81 % of samples, respectively. Interestingly, BPA showed lower detection frequency compared to BPS. These results are consistent with other studies performed on urine samples.
Limitations, reasons for caution: The limitation of the developed method is the time-consuming sample preparation and analysis cost.
Wider implications of the findings: These results document for the first time the presence of BPS in seminal fluid. Knowing the concentration of BPA and BPS in seminal fluid is crucial for mitigating the associated health risks and

initiating intervention and prevention strategies. Our future work will evaluate the influence of BPS concentration on spermatozoa.
Trial registration number: AZV NV18-01-00544; CZ.02.2.69/0.0/0.0/19_074/0012727

P-007 Effect of swim-up on sperm morphology

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Study question: To evaluate the effect of swim-up on the percentage of certain morphological defects in the semen population
Summary answer: Swim-up preparation led to significantly lower percentage of spermatozoa with cytoplasmic droplets, thick neck and also multiple defects.
What is known already: Swim-up is routinely used sperm preparation technique in ART practice. It is widely known that swim-up enhances sperm quality in terms of motility and sperm morphology. However, the effect of swim-up on the frequency of occurrence of the specific sperm morphological abnormalities is still missing.
Study design, size, duration: This observational study involved 30 teratozoospermic patients of Nadezhda Women's Health hospital between December 2020 and January 2021. Sperm morphology was evaluated before and after swim-up preparation.
Participants/materials, setting, methods: Native semen was liquefied and was subjected to swim up. Semen analysis performed according to WHO 2010. Native semen and swim up samples from the same men were subjected to Kruger strict morphological evaluation.

The analyzed sperm morphological defects included: head defects (large, small, tapered, pyriform, round, amorphous and double heads); midpiece defects (bent, asymmetrical, thin, thick, presence of cytoplasmic droplet); tail defects (short, hairpin, bent, coiled tail and terminal droplet) and multiple defects.
Main results and the role of chance: Wilcoxon paired test showed that the percentage of morphologically normal spermatozoa was significantly higher in the swim-up samples in comparison to the native semen (8.5±4.2% vs 4.9±3.2%, $p<0.05$). In addition, the percentage of spermatozoa bearing multiple defects was found to be significantly lower in the swim-up samples than in the native semen (25.8±11.6% vs 37.0±15.0%, $p<0.05$). Two specific sperm morphological defects were found to be significantly lower after swim-up preparation: the presence of cytoplasmic droplets (6.0±1.0% vs 8.6±1.5%, $p<0.05$) and the thick neck (9.7±5.5% vs 12.8±5.8%, $p<0.05$). No significant different were observed in the other morphological defects between swim up samples and native semen ($p>0.05$).
Limitations, reasons for caution: Results obtained from this study need to be confirmed by larger group of samples.
Wider implications of the findings: Our study showed a significant reduction of certain midpiece defects after swim-up. The observed selection of spermatozoa without thick necks and cytoplasmic droplets explains the effectiveness of swim-up on ART. In addition, the obtained results can serve as a guide for future validation of new sperm preparation techniques.
Trial registration number: Not Applicable

P-008 The diameter of standard seminiferous tubules varies with the different histological patterns in patients with non-obstructive azoospermia

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Study question: Does the standard, not-dilated seminiferous tubules (STs) diameter vary according with different testis histology patterns in patients with non-obstructive azoospermia (NOA)?
Summary answer: The standard STs diameter differed significantly among cases with different testis histology: hypospermatogenesis (HYPO) had the highest STs diameter compared to the other histological subgroups.
What is known already: During microdissection testicular sperm extraction (mTESE), the identification of dilated STs, as subjectively evaluated at high magnification by comparing their apparent caliber with that of the surroundings, is crucial to identify residual foci of intact spermatogenesis and to retrieve sperm. Previous studies have demonstrated that dilated STs contain sperm in most cases, but it is not clear why in some cases an apparent normal tubular caliber does not correspond to spermatogenesis integrity. Aim of the present study was to assess whether different histology patterns could affect the STs diameter.
Study design, size, duration: We retrospectively evaluated 168 patients with NOA undergoing unilateral (N=91) or bilateral (N=77) mTESE from 2018 through 2019. One or more biopsy samples representative of the overall appearance of the testicular parenchyma were taken from one (for unilateral mTESE) or both testes (for bilateral mTESE), was fixed in Bouin's solution and sent to the pathologist. Histological analysis was conducted by the same experienced pathologist, who examined at least 100 different tubule sections per biopsy sample.
Participants/materials, setting, methods: Each tubule section (N=100 per sample) was evaluated at 10X magnification with a micrometer to measure the tubule diameter, then the mean ST diameter was computed. The basal membrane (BM) thickening was evaluated in every section, and a score was assigned by multiplying the degree of BM fibrosis (mild=1, moderate=2, severe=3) for the number of sections (e.g. BM score for moderate fibrosis in 50 sections=2x50=100). Leydig cells hyperplasia (LCH), if present, was also annotated.
Main results and the role of chance: The median + interquartile range STs diameter was 140; 110-185 mm, while the median BM score was 100; 10-150. Sertoli cell only syndrome (SCO) was found in 51.1% of cases, focal SCO (FSCO) in 4,7%, early (EMA) and late (LMA) maturation arrest in 10.2 and 2.73% respectively, HYPO in 26.17% and hyalinosis (HL) in 5% of cases. LCH was found in 46.88% of samples. STs diameter, BM score and LCH differed significantly among the different histological patterns: STs diameter was 125; 100-148 in SCO, 162; 102-187 in FSCO, 130; 100-175 in EMA, 145; 130-195 in LMA, 205; 170-240 in HYPO and 57.5; 42.5-100 in HL. HYPO samples also had the lowest BM score (20;1-100; $p<0.0001$) and LCH prevalence (23.8%, $p<0.0001$) compared to the other histological subgroups (HS). A multinomial logistic regression for prediction of different histological subgroups was run with STs diameter, BM score and LCH as candidate predictors: the model explained the 29% of variability of the outcome and correctly classified 69% of cases. STs diameter significantly predicted FSCO (RR 1.02, 95% CI 1.0-1.04), LMA (RR 1.02, 95% CI1-1.04) and HYPO (1.03, 95%CI 1.02-1.04), while BM score significantly predicted HL (RR 1.07, 95%CI 1.02-1.13).
Limitations, reasons for caution: The STs were carefully cut before extraction in order to preserve their structural integrity, however the accuracy of such a method of estimating the STs diameter needs to be assessed by further studies.
Wider implications of the findings: The identification of dilated STs remains the best strategy to retrieve sperm by mTESE, since larger STs diameters are mostly associated with the more favorable histological patterns. However, dilated STs may be also found in cases with LMA, which explains why in some cases dilated STs do not contain sper.
Trial registration number: not applicable

P-009 A modified sperm chromatin dispersion test, LensHooke® R10, for quick and accurate determination of human sperm DNA fragmentation

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Study question: The performance and efficiency of the LensHooke® R10 test kit were evaluated by the clinical examination for precision, accuracy, and time.
Summary answer: The LensHooke® R10 based on sperm chromatin dispersion test offers not only quick testing for sperm DNA fragmentation but also reliable and accurate test results.
What is known already: Sperm chromatin dispersion (SCD) test, one of the most commonly used testing for sperm DNA fragmentation (SDF), can be conducted promptly and without the need for expensive laboratory instruments.

However, the main disadvantage of the SCD test is inter-observer variability in categorizing the size of characteristics halos surrounding the core of sperm. Moreover, it takes more than one hour to accomplish whole assay procedures making this testing an inefficient diagnostic tool. These may hinder its broad availability among andrology laboratories or prevent it from being routinely used for the evaluation of male infertility.

Study design, size, duration: A total of 108 participants was included in this prospective study. Data was collected from the reproductive medicine center between June and December 2020.

Participants/materials, setting, methods: This study included 108 consecutive male partners of couples attending for assisted reproductive treatment. SDF was simultaneously tested by using LensHooke® R10 (R10) and Halosperm® G2 (G2) respectively. We evaluated the correlation and agreement between two SCD-based test kits. The repeatability and reproducibility of the SCD kits were assessed by intra-and inter-observer agreement experiments. The sensitivity, specificity, positive predictive value, negative predictive value for the R10 was determined by receiver operator characteristics (ROC) curve analysis.

Main results and the role of chance: The R10 produced more clear sperm core and dispersed chromatin, therefore highly recognizable images can be easily and accurately categorized when scoring of SDF. It took 50% less time for SDF testing by the R10 compared to the G2 (38.26 ± 9.85 minutes vs. 76.52 ± 19.7 minutes, P< 0.0001). The SDF% results showed a strong correlation for the R10 and G2 with Spearman's coefficients of rank correlation (rho) above 0.8 (P<0.0001, N=108). The R10 showed 89.8% accuracy with 87.9% sensitivity, 90.8% specificity, 82.9% PPV, and 93.7% NPV on the measurement of SDF% at the threshold value of 22%. Intraclass correlation coefficients (ICC) >0.9 showed a strong agreement between two observers on the testing of SDF using the R10. ICC >0.9 showed a high intra-observer agreement within 4 repeated testing on SDF using the R10. The R10 showed an intra-observer's precision of coefficient variation, CV <10% for SDF%. In addition, SDF% test results obtained by the R10 for asthenospermic (31.8% ± 16.7%), teratospermic (22.9% ± 14.4%), and oligoasthenoteratozoospermic samples (36.6% ± 14.4%) were significantly higher than that observed in normozoospermic samples (15.3% ± 10.2%, p< 0.05), was comparable with the G2.

Limitations, reasons for caution: The sample size of 4 semen specimens used to evaluate the intra-and inter-observer agreement was a limitation. Besides, evaluating the relationship between the SDF and clinical outcome of ART is necessary for further study.

Wider implications of the findings: The new in vitro diagnostics reagent, LensHooke® R10, is a simple and quick test kit that offers reliable and accurate test results of sperm DNA fragmentation, can be routinely used in male infertility evaluation.

Trial registration number: CS2-20012

P-010 Timing of TESE does not affect laboratory outcomes

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Study question: What is the outcome of intracytoplasmic sperm injection (ICSI) using testicular spermatozoa obtained on the day of oocyte pick-up (OPU) or the day before OPU.

Summary answer: Testicular spermatozoa were obtaining the one day before OPU does not affect fertilization rate, top quality embryo on day 3, and blastocyst utilization rate.

What is known already: Usually, TESE is performed just before OPU. OPU is generally cancelled if no sperm is retrieved. The use of fresh testicular spermatozoa, obtained the day before OPU could offer the couple and the caring team both medical and practical advantages. The benefits of this approach, however, has not been evaluated in detail. An uncontrolled preliminary study has revealed that regular fertilization and pregnancy rates could be achieved with sperm extraction performed one day before OPU.

Study design, size, duration: This was a single-center retrospective study in Bahceci BIH IVF center. Sixty-six patients suffering from azoospermia from January 2015 to December 2020 were evaluated. TESE was performed either on the OPU day (43 patients; group A) or one day before OPU (23 patients; group B).

Participants/materials, setting, methods: In this study, primary outcomes were motile spermatozoa at ICSI, fertilization, top quality embryo on day 3, and blastocyst utilization rate. Statistical analyses were performed with chi-squared tests.

Main results and the role of chance: There is no statistical difference fertilization rate (72.3% vs. 72.2, p>0.05), top quality embryo rate on day 3 (58.3% vs 58.3%, p>0.05) and blast utilization rate (43.98% vs 49.58%, p>0.05) between group A and B, respectively.

Limitations, reasons for caution: The retrospective nature of this study may not eliminate potential bias. On the contrary, the strength of our study is that all procedures were performed by the same operators, so there are no operator-dependent differences. More research is needed to prove our findings.

Wider implications of the findings: TESE procedure can be performed one day before OPU without compromising success.

Trial registration number: 1

P-011 Automated sperm morphology assessment using artificial intelligence technology

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Study question: Can LensHooke X1 PRO semen analyzer be used to evaluate sperm morphology in men with infertility?

Summary answer: Morphology results generated by X1 PRO are highly reliable when normal sperm forms are ≥4% and therefore they can be reported in such cases .

What is known already: Most laboratories rely on manual evaluation of sperm morphology smears, which is a time-consuming procedure and its results are subjected to a relatively high variability. However, in recent years the computer-assisted semen analyzers are being increasingly used to evaluate sperm morphology. The X1 PRO semen quality analyzer was designed for in vitro diagnostic use to analyze sperm concentration, total, progressive and non-progressive motility as well as sperm morphology based on WHO 5th edition criteria. Evaluation of sperm morphology using X1 PRO based on AIOM (Artificial Intelligence Optical Microscopic)-based technology requires no fixation steps or staining unlike the manual method.

Study design, size, duration: This cross-sectional study used 31 semen samples from 8 normozoospermic healthy volunteers and 5 infertile men with a minimum abstinence period between 2 - 3 days. While the 8 healthy semen donors produced a total of 26 ejaculates, which were split into 88 aliquots, the 5 infertile patients produced 5 ejaculates that were split into 13 aliquots.

Participants/materials, setting, methods: A total of 101 aliquots were prepared from the native semen samples either by dilution or concentration using seminal plasma of the respective donors. Automated semen analysis was performed by the X1 PRO semen analyzer and the results of sperm morphology were compared with manual morphology results using Diff-Quik staining. Statistical analysis was carried out to calculate the positive predictive value (PPV) and negative predictive value (NPV) of X1 PRO semen analyzer.

Main results and the role of chance: The X1 PRO sperm morphology results show a weak non-significant (P=0.2441) correlation (r=0.119) with the manual results. However, X1 PRO demonstrated a high PPV (97.7%) and a low NPV (9.1%) for correct assessment of sperm morphology (≥4%) when compared to manual results. Due to its high PPV, laboratories can report the morphology results generated by X1 PRO in all such cases when normal sperm forms are ≥4%. However, a manual evaluation is necessary in patients with abnormal morphology (<4%).

Limitations, reasons for caution: One of the limitation of this study is that X1 PRO morphology values did not correlate with manual results. The low NPV seen in our study is due to the inclusion of very few samples with abnormal sperm forms (<4%) in the analysis.

Wider implications of the findings: The X1 PRO's combination of speed, ease of use, accuracy and portability makes it a good choice of device for small medical offices to large IVF centers. High PPV of X1 PRO allows it to correctly identify normal sperm forms for diagnostic use.

Trial registration number: 18-771

P-012 A new sperm preparation technique for ICSI

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Study question: Is there any difference in this technique(swim-in) according to clinical results in ICSI cycles?
Summary answer: Fertilization rates are significantly better with this new technique(swim-in). There is no difference in pregnancy and ongoing pregnancy rates between swim-in and DGS techniques.
What is known already: Sperm separation is crucial in assisted reproductive technologies, based on different principles like migration, filtration, or density gradient centrifugation techniques. However, there are some studies that centrifugation steps and using gradient solutions may increase sperm DNA fragmentation. On the other hand, multiple contacts to different plastic surfaces such as pipettes, plastic tubes, etc., and also pipetting the ejaculate in sperm preparation may have detrimental effects. The ideal sperm separation technique should be non-invasive, not time-consuming, easy, and cost-effective, not cause sperm damage or non-physiological alterations of the separated sperm cells, eliminate dead spermatozoa and other cells, including leukocytes.
Study design, size, duration: This is a prospective randomized study between 01/02/2019...01/01/2021. Sperms were prepared either with this new technique that we call "swim-in"(n=359) or with density gradient centrifugation(n=404) before microinjection (ICSI). Fertilization rates, clinical pregnancy rates, and on-going pregnancy rates were compared between groups. Sperm motility is less than %20 patients are not included in the study. t-test and Chi-square test used. p<0.05 significant.
Participants/materials, setting, methods: For this new centrifugation free technique(swim-in), a Braun injector, 21G green needle, and sperm washing medium are used. First, we pulled a 0.5 ml sperm wash medium in a Braun injector. After sperm liquefaction, we put Braun injector with the needle in the sperm cap perpendicularly and waited 30 minutes at 37-degree Celcius. Sperms swam through the needle to the injector used for ICSI. For DGC sperms were prepared according to the 2015 WHO manual. Main results and the role of chance: We try to make a centrifugation-free, gentle, cost-effective, and easy sperm preparation technique and called it a swim-in technique. Groups were comparable according to mean age, collected oocyte numbers, and oocyte maturation ratios. Fertilization rates are %73,2(1786/2438) vs %66,9(2167/3237) respectively for swim-in and DGC groups. Clinical pregnancy rates are %45,1 vs %42,3 respectively for swim-in and DGC groups. On-going pregnancy rates are %41,5 vs %39,6 for swim-in and DGC respectively. There is a significant difference between the two techniques according to fertilization rates but no difference according to clinical pregnancy and ongoing pregnancy rates.
Limitations, reasons for caution: Sperms can move by their own motility from ejaculate to the sperm washing medium for that reason with severe sperm problem patients are not suitable for this technique.
Wider implications of the findings: This technique is centrifugation free so there are no detrimental g-forces. The needle used may mimic the physiological environment like Uterine Tube. Alternative sperm washing mediums like follicular fluid or chemical attractor progesterone rich medium can be used. DNA fragmentation rates and live birth rates should be established for future aspect.
Trial registration number: not applicable

P-013 Sperm freezing does not affect live birth rates: results from 6,594 cycles in normozoospermic patients

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Study question: Does sperm cryopreservation influence the reproductive outcomes of normozoospermic patients undergoing elective ICSI?
Summary answer: After controlling for confounders, the use of cryopreserved semen from normozoospermic patients does not affect pregnancy and live birth rates after ICSI.
What is known already: Sperm cryopreservation with slow freezing is a common practice in ART. While frozen-thawed semen typically presents reduced motility and vitality, its use for ICSI is generally considered adequate in terms of reproductive outcomes. Nevertheless, most studies comparing reproductive outcomes between fresh versus cryopreserved sperm include patients with oligo- and/or asthenozoospermia, where the altered quality of the sample can partially mask the full effect of freezing/thawing. The objective of this study is to ascertain whether ICSI using fresh or cryopreserved semen from normozoospermic patients results in similar fertilization rates and reproductive outcomes.
Study design, size, duration: Retrospective cohort of 6,594 couples undergoing their first elective ICSI cycle between January 2011 and December 2019, using normozoospermic partner semen (fresh or cryopreserved). All cycles involved a fresh embryo transfer, either at cleavage or blastocyst stage. Cycles were divided in 4 groups: fresh semen with partner's oocytes (FSPO, n=1.878), cryopreserved semen with partner's oocytes (CSPO, n=142), fresh semen with donor oocytes (FSDO, n=2.413), and cryopreserved semen with donor oocytes (CSDO, n=2.161).
Participants/materials, setting, methods: A slow freezing protocol using GM501 SpermStore medium (Gynemed, Lensahn) was used for all sperm cryopreservation. Sperm washing, capacitation, and selection prior to ICSI were performed equally for fresh and frozen-thawed samples, using pellet swim-up in IVF® medium (Vitrolife, Göteborg). Fertilization rate (FR), pregnancy (biochemical, clinical, and ongoing) and live birth (LB) rates were compared among study groups using Pearson's Chi square and Student's t-test. A p-value <0.05 was considered statistically significant.
Main results and the role of chance: Male and female age, sperm concentration and motility after ejaculation, and number of oocytes inseminated were similar between study groups compared (FSPO vs. CSPO, FSDO vs. CSDO). As expected, oocyte donation cycles resulted in higher LB rate than cycles in which partner's oocytes were used (30.04% vs 18.17%, p<0.001). In cycles using partner's oocytes, no significant differences were observed between fresh and cryopreserved sperm in FR, pregnancy and LB rates (p>0.05 for all outcomes). However, in oocyte donation, the mean FR after ICSI using cryopreserved semen (73.6 ± 19.6) was lower than the FR obtained with fresh semen (75.1 ± 19.2), p=0.010. Similarly, in oocyte donation cycles, the biochemical pregnancy rate was significantly lower when using cryopreserved semen (48.5% in CSDO vs. 52.3% in FSDO, p=0.009), while clinical, ongoing pregnancy and LB rates were similar between both semen status (p>0.05). In oocyte donation, a subgroup analysis including only the ICSI cycles with embryo transfer at blastocyst stage (n=1.187 for FSDO, n=337 for CSDO) confirmed that the LB rate was comparable between fresh and cryopreserved semen groups (34.7% vs 35.6% respectively, p=0.76), without significant differences in pregnancy rates neither (p>0.05 for all outcomes).
Limitations, reasons for caution: Caution should be exerted when extrapolating these results to different protocols for sperm cryopreservation and selection, or to IVM and classical IVF cycles, which were excluded from analysis. Due to the retrospective nature of the study, some uncontrolled for variables may affect the results.
Wider implications of the findings: Sperm cryopreservation does not affect pregnancy and live birth rates in normozoospermic patients, although it may lower slightly fertilization rates. In line with previous studies including patients with an apparent male factor detected after routine semen analysis, sperm cryopreservation is a safe and convenient technique.
Trial registration number: not applicable

P-014 Effect of microsurgical varicocelectomy on fertility outcome and treatment plans of patients with severe oligozoospermia: An original report and meta-analysis

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Study question: Does microsurgical subinguinal varicocelectomy (MSV) improve semen parameters and fertility outcomes of patients with severe oligozoospermia (SO) and clinical varicocele?

Summary answer: MSV significantly improves semen parameters of patients with SO and can broaden their fertility treatment options. What is known already: Varicocele ligation has been proven to restore semen parameters and improve pregnancy rates in men with clinically palpable disease. However, its effect in men with SO is less clearly elucidated. Patients with SO are candidates for in vitro fertilization and intracytoplasmic sperm injection. Improvements in semen quality following varicocele ligation in this patient population may broaden their fertility options. While few studies indicate an improvement in semen parameters, reports revealing a negative outcome following surgery in this patient group were also published.

Study design, size, duration: This original report and meta-analysis examined the impact of MSV on semen parameters and fertility outcomes of men with SO. A retrospective chart review of 85 patients was conducted on patients with SO who underwent MSV. A literature search was carried out according to the PRISMA guidelines using the key words "severe oligozoospermia" and "varicocele". 8 scientific articles (including the current study) reporting the impact of MSV on men with SO were included.

Participants/materials, setting, methods: Changes in semen parameters postoperatively were compared with pre-operative results. The reported natural pregnancy rates were also calculated. The Wilcoxon signed-rank test was used to compare semen and hormone values before and after varicocelectomy. The Chi-squared test was used to assess the changes in TMSC groups after surgery. The meta-analysis was performed using comprehensive meta-analysis software (Biostat, Englewood, NJ, USA). Statistical significance was set at =0.05. The random-effects model was used to adjust for heterogeneity.

Main results and the role of chance: The original study reported significant improvements in sperm concentration ($p < 0.001$), total motility ($p = 0.003$), progressive motility ($p = 0.002$) and TMSC ($p < 0.001$) was following the surgery. in semen parameters following surgery. 78 patients had a pre-operative TMSC < 5 million. Following surgery, 9 (11.5%) patients had a TMSC between 5-9 million, while 14 (17.9%) patients had a TMSC > 9 million. The meta-analysis shows a statistically significant increase in sperm count following surgery (MD 5.64, 95% CI, 4.195-7.090, $p = 0.00$) with an acceptable degree of heterogeneity (Q value= 8.75, p= 0.188, I^2= 31.5%). Similarly, the total motility significantly increased by 7.77% (p= 0.001) following surgery (95% CI, 3.248-12.297), however, with considerable heterogeneity among the reported results (Q value= 34.4, p< 0.001). TMSC was assessed by three studies, including ours. The meta-analysis shows a significant increase in TMSC following surgery (MD 8.44 million sperm, 95% CI, 4.648-12.228, p <0.001) (Q Value= 2.53, p= 283, I^2=20.7%).

A total of 6 studies reported the natural pregnancy rate of patients with SO who underwent surgery. Out of 530 patients with preoperative SO, 146 patients achieved natural pregnancy following surgery indicating that the reported pregnancy rate was 27.5%.

Limitations, reasons for caution: One limitation to the original study is its relatively small sample size. However, this was compensated by conducting a meta-analysis and reporting the outcome of 601 patients with SO who underwent varicocele ligation. Another limitation is the retrospective nature of the study design.

Wider implications of the findings: 29.5% of SO patients in the original study became eligible for IUI following varicocelectomy. Meta-analysis showed that 27.5% of patients achieved natural conception following surgery. Such information is beneficial during patient counselling and needs to be measured against the financial and clinical implications in order to make sound treatment decisions.

Trial registration number: NA

P-015 Characterization of ultrastructural morphology of human sperms by field-emission scanning electron microscopy using the NanoSuit method

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Study question: Can the NanoSuit method to observe sperm cells in wet conditions help treat male infertility using a field emission scanning electron microscope (FE-SEM)?

Summary answer: Compared with the conventional fixation method, the NanoSuit method can easily prepare FE-SEM samples without causing contraction and denaturation of human sperm cells.

What is known already: Evaluation of sperm morphology by optical microscopy is important for identifying male infertility. FE-SEM observation is useful for a more detailed evaluation of sperm morphology; however, a lot of the morphological information of the cells is lost by chemical fixation, dehydration, and freeze-drying. The NanoSuit method enables FE-SEM observation of unfixed cells under a high vacuum environment by electron beam polymerization of extracellular substances called NanoSuit. It has been reported that a sample prepared by the NanoSuit method retains the morphological information of live cells better than a sample prepared by the conventional fixation method.

Study design, size, duration: This laboratory study was conducted with informed consent and IRB approval. Semen parameters were within the WHO normal reference range.

Participants/materials, setting, methods: The conventional fixation method sample was prepared by fixing (glutaraldehyde and osmium), dehydration (ethanol and t-butyl alcohol), and freeze-drying. The NanoSuit method sample was introduced into the FE-SEM directly without conducting the above treatments. For observation, a JSM-7100F (JEOL, Japan) was used at an acceleration voltage of 1.0 kV. The vacuum level of the observation chamber was 10^{-3} to 10^{-6} Pa.

Main results and the role of chance: Sperm head segmentation (acrosome, equatorial segment, and post acrosome), midpiece, and tail including endpiece could be clearly identified in the FE-SEM sample prepared by the NanoSuit method. Transmission electron microscopy revealed the existence of a thin polymerized extra layer, the NanoSuit, on the surface of the sperm. It is suggested that the presence of the NanoSuit layer enables FE-SEM observation of the unfixed sperm. The conventional fixation method causes a statistically significant contraction in the sperm head size compared to that calculated from optical micrographs (13.5 μm^2 vs. 11.6 μm^2, $p<0.001$). Furthermore, wheat germ agglutinin (WGA), a lectin, which is known to have the ability to bind to the sperm surface, did not bind to the fixed FE-SEM samples. This means that the original cell surface properties are lost in the fixed sperm sample. On the other hand, the FE-SEM sample prepared by the NanoSuit method did not show a statistically significant contraction of the sperm head compared to that calculated from optical micrographs (13.2 μm^2 vs 12.9 μm^2, $p=0.416$); it also revealed a detailed binding pattern of gold-labelled WGA to the sperm surface. These results indicate that the NanoSuit method can prepare FE-SEM samples without sperm contraction and denaturation.

Limitations, reasons for caution: Characteristic sperm morphology in patients with male infertility should be investigated in future studies.

Wider implications of the findings: The NanoSuit method does not use chemical carcinogens and can prepare an FE-SEM sample in a shorter time than the conventional fixation method. The evaluation of ultrastructural morphology of unfixed sperms by this method may be useful for the identification of new morphological features and the evaluation of male infertility.

Trial registration number: not applicable

P-016 Using artificial intelligence to predict semen upgrading after microsurgical varicocele repair

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Study question: Can we use artificial intelligence models to predict semen upgrading after microsurgical varicocele repair?

Summary answer: A machine learning model performed well in predicting clinically meaningful post-varicocelectomy semen upgrade using pre-operative hormonal, clinical, and semen analysis data.

What is known already: Varicocele repair is recommended in the presence of a clinical varicocele together with at least one abnormal semen parameter,

and male infertility. Unfortunately, up to 50% of men who meet criteria for repair will not see meaningful benefit in outcomes despite successful surgery. Nomograms exist to help predict success, but these are based out of single-center databases, do not incorporate hormonal data, and are rarely designed to predict pre-defined, clinically meaningful improvements in semen parameters.

Study design, size, duration: Data were collected from an international, multi-center retrospective cohort. A total of 240 men were identified. Data from 160 men from Miami, USA and 80 men from Toronto, Canada were included. Data was collected from 2006 to 2020.

Participants/materials, setting, methods: We collected pre and postoperative clinical data following varicocele surgery. Clinical upgrading was defined as an increase in sperm concentration that would allow a couple to access new reproductive technologies/techniques. The tiers used for upgrading were 0-1million/cc (Intracytoplasmic Sperm Injection), 1-5 million (In Vitro Fertilization), 5-15 million (Intrauterine Insemination), and >15 million (Natural conception). Artificial intelligence models were trained and tested using R to predict which patients upgraded after surgery.

Main results and the role of chance: 51% of men underwent bilateral varicocele repair. The majority of men had grade 2 varicocele on the left, and (when present) a grade 1 varicocele on the right. Overall, 47% of men experienced an upgrade following varicocele surgery, 47% did not change, and 6% downgraded. The data from Miami were used to create a random forest model for predicting clinically significant upgrade in sperm concentration. The most informative model parameters were preoperative FSH, sperm concentration, and surgical laterality. The model identified three clinical categories: men with unfavorable, intermediate, and favorable features to predict varicocele upgrade. On external validation using data from Toronto, the model accurately predicted upgrade in 87% of men with favorable features, and in 49% and 36% of men with intermediate and unfavorable features, respectively. Overall, the model performed well on external validation with an AUC of 0.72 and good calibration.

Calibration plots, using cross-validation, define how well the predicted probabilities match the actual probability of sperm concentration upgrade. The random forest model was run twelve times. All model characteristics are the mean of ten model runs with the highest and lowest performing runs removed.

The model was translated to an online calculator that can be used by clinicians.

Limitations, reasons for caution: One limitation to our study is that we were not able to predict total motile sperm count (TMSC), which has been shown to perform slightly better than concentration at predicting assisted reproduction outcomes. By focusing on clinically significant upgrading, this difference should be minimized.

Wider implications of the findings: Predicting the chances of clinically significant semen upgrading after varicocele repair is essential for patients and clinicians to understand. Several men undergo surgery with no subsequent benefit, which may lead to a delay in definitive treatment with IVF/IUI. Understanding their chances will help couples make better informed decisions moving forward.

Trial registration number: not applicable

P-017 The maintenance of testicular architecture and germ cell in adult testis tissue under organ culture condition based on the gas-liquid interface method

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Study question: Can the gas-liquid interface organ culture system that achieved in vitro spermatogenesis in mice also support in vitro spermatogenesis in human adult testis?

Summary answer: Although the progression of spermatogenesis was not observed, germ cells were maintained without the degeneration of the architecture in both fresh and cryopreserved testicular tissues.

What is known already: Although the research on in vitro spermatogenesis have been conducted for 100 years, only the organ culture system using gas-liquid interface method achieved in vitro spermatogenesis in mice. It has not been verified whether this culture system can be applied to other mammals including humans and induce spermatogenesis.

Study design, size, duration: Testicular tissue was obtained from the transgender patients receiving sex reassignment surgery. Testicular specimens were either immediately processed for cultivation or cryopreserved, using a vitrification freezing protocol. Organ culture of testicular fragments was performed in three different media for a maximum period of 3 weeks to evaluate the short-term changes in the cultured tissues (viability, proliferation and maintenance of germ and somatic cells).

Participants/materials, setting, methods: Fresh and cryopreserved-thawed testis fragments (1–2 mm^3) were cultured using the organ culture system in alpha-MEM with knock-out serum replacement (K group), alpha-MEM with lipid-rich BSA (A group) and DMEM with FBS (D group). Luteinizing hormone, follicle stimulating hormone and testosterone were supplemented. The number of germ cells (using DDX4), proliferative activity of germ cells (using EdU assay) and intratubular cell apoptosis (by TdT-mediated dUTP Nick End Labeling) were evaluated by immunohistochemical staining weekly.

Main results and the role of chance: The architecture of the seminiferous tubules was maintained until the second week of culture in both the fresh and the cryopreserved culture group. The number of DDX4-positive germ cells per seminiferous tubule in groups D, K, and A was 49 ± 24, 55 ± 21, 50 ± 26 cells/tubule in 1 day, 32 ± 13, 42 ± 7, 36 ± 21 cells/tubule in 1week, respectively. The numbers gradually decreased to 26 ± 8, 24 ± 6 and 27 ± 18 cells/tubule, in 2 weeks, respectively, with no difference among the groups. The number of intratubular EdU-positive cells of groups D, K, and A was 0.2 ± 0.2, 2.8 ± 2.1, 1.1 ± 0.8 cells/tubule at 1 day, 0.1 ± 0.2, 0.5 ± 0.6, 0.3 ± 0.6 cells/tubule at 1 week, respectively. The values were 0.01, 0.05, and 0.03 at 2 weeks. Thus, EdU-positive cells drastically decreased from the first week of culture. The number of DDX4-positive germ cells and the intratubular EdU-positive cells in the cryopreserved culture group was not different from that in the fresh culture group.

Limitations, reasons for caution: Current organ culture systems are incomplete, being unable to induce human in vitro spermatogenesis. Further research is needed to improve culture condition with the aim of producing fertile sperm of infertile adult male patients.

Wider implications of the findings: Our organ culture system could maintain testis structure and germ cells. By using the testis tissues of the transgender patients, which are available with their consent, we will promote the investigation of the culture condition necessary for germ cell proliferation and differentiation.

Trial registration number: Grant-in-Aid for Scientific Research on Innovative Areas 18H05546, Grant-in-Aid for Young Scientists (A) 17H05098 and Takeda Science Foundation

P-018 Observational study on the behaviour of the seminal sample in donors: seasonal variation according to parameters

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Study question: What is causing the decline in semen quality worldwide?

Summary answer: Our results indicated significant differences, finding a decrease in sperm concentration/mobility in summer compared to the other seasons, with greater differences being observed in spring.

What is known already: Infertility is an increasing global problem and it is estimated that approximately 15 to 20% of all couples experience it at some point in their reproductive life. Among all causes, the male factor is becoming increasingly important as seminal quality is steadily declining globally. Knowing that the spermatogenesis process is very sensible to temperature fluctuations we could focus on the ambient temperature as one of the causes.

Study design, size, duration: Taking into account that donors are selected for having an optimal seminal quality, a retrospective study (January 2006 / February 2020) was proposed at IVI Alicante from 160 seminal samples to determine whether environment actually affects spermatogenesis and semen quality. It was evaluated whether there is variation in donors in mobility/volume / concentration depending on the season and quarter of the year in which the sample was obtained; and relation to the age of the donor.

Participants/materials, setting, methods: The inclusion criteria were sperm donors between 18 and 35 years old, anonymously, with good physical health, full capacity to act, and with seminal samples with characteristics to survive sperm thawing, complying with the requirements according to Spanish law on ART 2006. Exclusion criteria were based mainly on poor sperm survival after thawing. The statistical analysis was performed with the R statistical software, version 4.0, linear and multiple regression, establishing significant differences when p <0.05.
Main results and the role of chance: The results indicated significant differences, finding in summer a decrease in concentration and sperm motility (p <0.05) compared to autumn and winter and obtaining the best quality in spring. The concentration and mobility decrease from 63.4 million sperm / ml and 49.6% in spring to 44.4 million (p = 0.009) and 39.9% mobility in summer (p = 0.0075). We found the same results comparing them by quarters, having 62.7 million / ml and 49.3% between April-June, up to 44.9 million (p = 0.003) and 39.3% mobility between July-September (p = 0.03), showing that July and September there is a decrease in both concentration and mobility. This association has not been significantly affected by age. Thus, we conclude that high temperatures affect seminal concentration and mobility.
Limitations, reasons for caution: More studies can be done to increase the number of donors and confirm our findings.
Wider implications of the findings: According to other studies, carried out in Denmark, Israel and China, with different latitudes and temperature-humidity, the same trend has been observed in sperm quality, decreasing the quality in summer and being optimal in spring-winter, so the temperature could be a variable to take into account when studying semen.
Trial registration number: Not applicable for non clinical-trials

P-019 Sperm parameter and ICSI / IVF outcomes after sperm selection using microfluidic sperm separator and density gradient centrifugation with swim-up in split semen sample

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Study question: To analyze whether microfluidic sperm selection (MSS) by ZyMōt™ improves sperm DNA fragmentation rate and embryonic development compared to density gradient centrifugation with swim-up (DGCS).
Summary answer: MSS by ZyMōt™ selects sperm for clinical use with less DNA damage significantly compared to DGCS.
What is known already: Conventional sperm preparation methods, such as density gradient centrifugation and the swim-up method utilize centrifugation during processing, may damage the sperm. MSS may allow for improved selection of normal sperm compared with conventional sperm preparation as it yields sperm with a lower DNA fragmentation rate. However, there are few clinical studies by sibling oocytes study compared to DGCS.
Study design, size, duration: This prospective study was performed between March 2020 and May 2020 at a reproductive center. All patients involved gave written consent, and institutional review board approval was granted. A total of 575 metaphase II oocytes were collected from 49 cycles. Wife's age was 34.7 ± 3.9 years old. Raw sperm concentration and motile sperm concentration was 63.1 ± 78.7M/mL, and 41.6 ± 67.7M/mL, respectively.
Participants/materials, setting, methods: Patients who performed ART for the first or second time were divided into two groups according to MSS and DGCS. Sperm DNA fragmentation rate (SDFR) and motile sperm concentration were compered between MSS and DGCS. SDFR was measured by sperm chromatin structure assay (SCSA) using a flow cytometer. Sibling oocytes were randomized into MSS-IVF, DGCS-IVF, MSS-ICSI, and DGCS-ICSI. Rate of two pronuclear (2PN) oocytes, blastocysts development, and good-quality blastocysts were compared between each group.
Main results and the role of chance: SDFR was 13.5 ± 11.8% for raw semen. SDFR was significantly lower after MSS (3.6 ± 4.1%) than that for raw semen and after DGCS (17.4 ± 14.8%) (P <0.01). Motile sperm concentration after MSS (19.0 ± 28.3M/mL) was significantly higher after than after DGCS (15.4 ± 15.3M/mL) (P<0.01). The number of IVF performed was 145 for MSS and 132 for DGCS. IVF results (MSS vs DGCS) were 2PN rate (73.1% vs 72.0%), blastocysts development rate (65.3% vs 55.4%), and good quality blastocysts rate (43.2% vs 34.9%). The number of ICSI performed was 149 for MSS and 149 for DGCS. ICSI results (MSS vs DGCS) were 2PN rate (77.9% vs 79.2%), blastocysts development rate (68.8% vs 65.8%), and good quality blastocysts rate (35.8% vs 30.6%). No significant difference was observed between MSS and DGCS for each parameter both IVF and ICSI.
Limitations, reasons for caution: The participants were limited to those who collected semen of 2mL or more and motile sperm concentration of above 1M/mL, because semen sample needed to be divided to MSS and DGCS.
Wider implications of the findings: This is the first study to conducted in sibling oosytes study with MSS and DGCS, in both IVF and ICSI. MSS is effective in collecting sperm with less DNA damage compared to DGCS. Motile sperm concentration after using MSS is sufficient to perform IVF as well as DGCS.
Trial registration number: not applicable

P-020 AZFc partial microdeletions of the Y chromosome is associated with severe oligozoospermia among Iranian men

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Study question: Are AZFc partial deletions correlated with severe oligozoospermia in Iranian men? Can we consider them as risk factors for infertility?
Summary answer: The frequency of total partial AZFc microdeletions was significantly higher in the oligozoospermia group compared to control group (8% vs. 3%, P = 0.028).
What is known already: Among many factors affecting male infertility, the second most common genetic factor is Y chromosome microdeletion. Some studies on partial AZFc microdeletions (especially on three major types; gr/gr, b1/b3 and b2/b3) have associated them with impaired spermatogenesis (azoospermia and oligozoospermia) in infertile men from different ethnicities. This finding is attributed to differences in alterations in pattern of *DAZ/CDY1* copy numbers as spermatogenesis related genes.
Study design, size, duration: 200 oligozoospermic (sperm count <5 mil./mL) and 200 fertile men were included as case and control groups, respectively. Individuals with karyotype abnormalities, complete microdeletions in AZF regions, infections, hypogonadism, history of chemotherapy and radiation, cryptorchidism or history of orchiopexy were not included. The study was approved by the Royan Institute Ethics Committee. Written informed consents were obtained from each participant.
Participants/materials, setting, methods: Total DNA from peripheral blood was used to amplify six sequence-tagged sites (STS) markers through multiplex PCR to detect AZFc partial deletions according to previous studies. Patterns of deletion in *DAZ* and *CDY1* copies were determined through PCR- RFLP.
Main results and the role of chance: The frequency of AZFc partial microdeletions was 8% in oligozoospermic men (16/200) which was significantly higher compared to 3% in control group (6/200) (P = 0.028). Hence, partial deletions may be considered as a risk factor for the male infertility in Iranian population. Also, gr/gr showed a higher frequency in oligozoospermic group (4%) compared to controls (1.5%) (P=0.126). The combination of *DAZ1/2+CDY1b* was the most observed deletion pattern in 8 oligozoospermic men with gr/gr deletion (75%), while among 3 controls with gr/gr, *DAZ3/4+CDY1a* (2 out of 3) and *DAZ3/4+CDY1b* (1 out of 3) were detected. Therefore, *DAZ1/2+CDY1b* can be correlated to oligozoospermia.
Limitations, reasons for caution: In order to achieve stronger statistical results, a larger sample size is of more help.
Wider implications of the findings: Risk of vertical transmission to male offspring and expansion in the size of deletions should be considered when providing ART services to infertile men. Genetic counseling is suggested in oligozoospermic men.
Trial registration number: -

P-021 An accurate and reliable screening for SARS-CoV-2 in human sperm samples by RT-PCR: A requirement to evaluate the viral contamination risk during SARS-CoV-2 pandemic

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Study question: How to ensure a reliable and accurate detection of SARS-CoV-2 in seminal plasma and spermatozoa fractions of human sperm samples?
Summary answer: This RT-PCR assay showed high sensibility, repeatability and reproducibility for SARS-CoV-2 detection in seminal plasma and spermatozoa fractions, with a detection limit of 17 genomes/reaction.
What is known already: SARS-CoV-2 pandemic brings numerous concerns, such as the safety of gametes for patients undergoing assisted reproductive technologies, fertility preservation or sperm donation. Transient viremia and expression of SARS-CoV-2 receptors in testis and accessory glands bring the question of the presence of the virus in sperm samples. Moreover, the contamination during sperm collection may be possible. The few available studies about this issue mostly showed the absence of SARS-CoV-2 detection in semen of COVID-19 patients, except one reported study. All these studies performed SARS-CoV-2 detection with RT-PCR assays approved for naso-pharyngeal swabs, without a process specifically validated for semen fractions.
Study design, size, duration: Method validation was conducted between July 2020 and January 2021. SARS-CoV-2 direct detection was performed according to the French Society of Microbiology guidelines (SFM). Repeatability (n=6), reproducibility (n=3), limit of quantification (n=2) and of detection (n=6) were evaluated in seminal plasma (SP) and spermatozoa samples isolated after density gradient centrifugation and cryopreserved. In addition, variability of the whole analytical method efficiency was evaluated in samples of men with normal (n=6) or altered sperm parameters (n=6).
Participants/materials, setting, methods: Samples were surplus semen obtained from men undergoing routine semen analysis after granting informed consent. Assays were performed on SP and frozen spermatozoa fractions. After automated RNA extraction (MGISP-960, MGI-Tech®), real-time RT-PCR was performed using the one-step multiplex TaqPath COVID-19 kit (ThermoFisher®) targeting three viral regions (ORF1, nucleocapsid-N and spike-S proteins). An exogenous internal control was added before RNA extraction. Positive samples and dilution ranges were prepared with a standard (SARS-CoV-2 inactivated virus, QnosticTM Randox®).
Main results and the role of chance: RT-PCR assay applied for human sperm samples has been previously validated and is routinely used for SARS-CoV-2 detection in naso-pharyngeal swabs. We evaluated the efficiency of RNA extraction and RT-PCR for SARS-CoV-2 detection in semen fractions. The qualitative and quantitative performance of the whole analytical method was validated with an accuracy profile for SP and spermatozoa fractions. Overall, for repeatability, the standard deviation (SD) of the cycle threshold (Ct) was lower than 0.40 for the strong positive sample and 0.50 for the low positive one. An exception was observed for the S target of the low positive SP samples (SD=3) which was consistent with S being the less sensitive target of the assay. For reproducibility, SD of the Ct was lower than 0.30 for the strong positive sample and 0.80 for the low positive, except for the S target of the low positive (SD=1.5). The linearity range was determined for N target, the most sensitive target of the RT-PCR assay. It layed between 5200 and 52 SARS-CoV-2 genomes/reaction. The limit of detection of the RT-PCR assay was 17 viral genomes/reaction. Equal efficiency of the assay was observed for SP and spermatozoa independently of semen parameters (normal and altered sperm parameters). Limitations, reasons for caution: Our detection method was validated for the whole process: RNA extraction (reagents and system), RT-PCR (reagents and thermocycler QuantStudio 5™) and for both SP and frozen spermatozoa fractions. Variability might be observed with a different extraction system or a different type of biological sample.
Wider implications of the findings: This validated RT-PCR assay enables accurate and reliable screening of SARS-CoV-2 in SP and spermatozoa fractions, mandatory to investigate the presence of the virus in semen samples of patients undergoing assisted reproductive techniques, fertility preservation or sperm donation, and to ensure viral safety in the cryobanking process during covid-19 pandemic.
Trial registration number: EudraCT 2020-A01409-30

P-022 Improvement in sperm concentration and motility after oral antioxidant supplementation in infertile men with varicocele who have not undergone surgical repair: systematic review and meta-analys

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Study question: Does oral antioxidant supplementation improve sperm quality in infertile men with varicocele who have not undergone surgical repair?
Summary answer: Oral antioxidant supplementation improves sperm concentration and motility in infertile men with varicocele who have not undergone surgical repair. What is known already: Benefit from oral antioxidant supplementation has been shown in infertile men with varicocele following surgical repair. Similarly, oral antioxidant supplementation has been suggested in infertile men with varicocele who have not undergone surgical repair. However, its effect currently remains controversial.
Study design, size, duration: A literature search was performed until January 2021 aiming to identify prospective studies evaluating the use of oral antioxidant supplementation alone or in combination in men with varicocele who have not undergone surgical repair.
Participants/materials, setting, methods: Seven prospective studies were identified, published between 1987 and 2018, including 278 infertile men with varicocele who had not undergone surgical repair. The number of patients included ranged from 20 to 65. Sperm analysis, evaluating sperm concentration, motility and morphology was performed in these studies before and after oral antioxidant supplementation. Meta-analysis of weighted data was performed using random effects model. Results are reported as weighted mean difference (WMD) with 95% confidence interval (CI).
Main results and the role of chance: Seven studies were included in the systematic review. Oral antioxidant supplementation was performed by a combination of pentoxifylline, zinc and folic acid (single study), a combination of l-carnitine, fumarate, acetyl-l-carnitine, fructose, CoQ, vitamin C, zinc, folic acid and vitamin B12 (single study), a combination of L-Carnitine, vitamin C, coenzyme Q10, vitamin E, vitamin B9, vitamin B12, zinc , and selenium, l-carnitine (single study), or sole treatment with acetyl-l-carnitine (single study), L-Carnitine (single study), Coenzyme Q10 (single study) or zinc sulfate (single study). For the purpose of meta-analysis, the effect of oral antioxidant supplementation was evaluated after three months of treatment.

Oral antioxidant supplementation significantly increased sperm concentration (WMD +5.65x106/ml 95% CI: +1.11 to +10.12 p=0.01, random effects model) and motility (WMD +4.30%, 95% CI: +0.86 to +7.74 p=0.01, random effects model) in infertile men with varicocele who had not undergone surgical repair.

On the other hand, no significance difference was observed in sperm morphology (WMD +3.9%, 95% CI: -0.16 to +8.04 p=0.06, random effects model) and volume (WMD +0.53ml, 95% CI: 0.0 to +1.0 p=0.052, random effects model).
Limitations, reasons for caution: The number of relevant trials and that of patients included is small to allow for solid conclusions to be drawn. Moreover, although different oral antioxidants have been administered in infertile who had not undergone surgical repair, subgroup analysis was not feasible.

Wider implications of the findings: Currently, limited evidence supports the use of oral antioxidants in the treatment of men with varicocele, who have not undergone surgical repair. Although the benefit in sperm concentration and motility appears to be modest, it might be important regarding achievement of pregnancy in these men.
Trial registration number: not applicable

P-023 Innovative ultra-rapid vitrification method for five or fewer testicular spermatozoa from non-obstructive azoospermic men after microsurgical testicular sperm extraction (Micro-TESE)

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Study question: What technique can be used to successfully cryopreserve five or fewer testicular spermatozoa from non-obstructive azoospermic men?
Summary answer: This method for cryopreserving five or fewer spermatozoa from non-obstructive azoospermic men showed a recovery rate above 90% and a survival rate of about 70%.
What is known already: Clinical outcomes of ICSI when using only five or fewer testicular spermatozoa after cryopreservation have been unsuccessful and are considered to be inferior to those using testicular fresh spermatozoa from Micro-TESE. A possible cause of these poor results has been the lack of a successful freezing technique. In these cases, repeated Micro-TESE and simultaneous oocyte pick up has been the only available treatment.
Study design, size, duration: Evaluation of the efficiency of cryopreservation by modified permeable cryoprotectant-free vitrification method (HTF supplemented with 0.1M sucrose and 10% SPS) for five or fewer testicular spermatozoa from 113 non-obstructive azoospermic men using Micro-TESE was conducted retrospectively at St. Mother Clinic between 2011 and 2018.
Participants/materials, setting, methods: This study included 113 non-obstructive azoospermic men. Each motile spermatozoon was carefully aspirated tail first into the pipette, put into a 2-µl microdroplet media of the vitrification medium near the tip of the Cryotop (Kitazato Corporation, Tokyo, Japan) submerged in liquid nitrogen vapor for 2 min and then immediately plunged in liquid nitrogen. The vitrified spermatozoa were warmed by dipping them into a droplet media. Successfully recovered motile sperm were selected and used for ICSI.
Main results and the role of chance: Number of patients, transfer cycles and collected sperms were 113, 192 and 560. Mean age of patients and their wives were 32.0±3.7y and 28.4±5.8y. Clinical pregnancy rate, miscarriage rate, live birth rate and number of live offspring were 24.0% (46/192), 19.6% (9/46), 19.3% (37/192) and 37 (Male: Female = 17: 20). Sperm recovery rate and survival rate were 90.3% (506/560) and 70.4% (356/506). Fertilization rate and mean number of transferred embryos were 51.6% (99/192) and 1.73 (1-2). Mean gestational weeks and mean body weight at birth were 39.23±5.27w and 2852.31±314.28g. No congenital anomalies were observed in any of the babies.
Limitations, reasons for caution: The maximum number of spermatozoa to which this method can be applied successfully is about 10. When the number of aspirated spermatozoa is over 10, some of them change direction and reach the mineral oil, and once this happens, they cannot be expelled out of the pipette.
Wider implications of the findings: This technique is very useful for the cryopreservation of very small numbers of testicular spermatozoa (fewer than 10) in order to avoid or reduce Micro-TESE interventions.
Trial registration number: N/A

P-024 Health and lifestyle detrimental conditions may impact the association between patient age and semen parameters in male populations

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Study question: Can health and lifestyle detrimental conditions impact the relationship between paternal age and semen quality parameters in a male population?
Summary answer: Health and lifestyle detrimental conditions can attenuate the negative relationship between age and sperm concentration in a male population.
What is known already: Paternal age has increased in parallel with maternal age but its contribution in couple subfertility deserves further investigation. Previous studies suggest that paternal ageing is associated with reduced semen volume and impaired sperm morphology and motility, but not with reduced sperm concentration. Several health and lifestyle conditions such as diabetes, hypertension and smoking can negatively affect semen quality. Since the distribution of these conditions is not homogeneous throughout men's reproductive life, one can hypothesize that their presence may confound the association between paternal age and semen quality parameters in male populations.
Study design, size, duration: This is a retrospective study with data from 5565 men examined in a single fertility clinic between 2015 and 2020. The impact of health and lifestyle conditions was assessed by comparing the effects of age on semen parameters in two different patient populations: the overall patient population and a subpopulation excluding patients with detrimental health or lifestyle characteristics, both divided in 4 age groups (A: 25-34, B: 35-39, C: 40-44 and D: ≥45 years).
Participants/materials, setting, methods: The study includes 5565 men aged 24 to 72 years providing semen samples to assess volume, progressive motility and concentration (WHO) in a single fertility clinic. Patients presenting diabetes, heart/circulatory diseases, andrological disorders, genital neoplasms, cystic fibrosis, Y microdeletions, abusive alcohol intake, smoking and/or recreational use of drugs were excluded from the healthy subpopulation. The effect of age on semen parameters was assessed by ANOVA (motility and volume) or Kruskal-Wallis one-way ANOVA (concentration). Main results and the role of chance: Of 5565 men included in the study, 2150 (38.6%) did not present any of the detrimental health and lifestyle conditions described above. In the overall patient population, semen volume [Mean±SD (mL); A: 3.14 ± 1.55, B: 3.01 ± 1.53, C: 2.83 ± 1.52, D: 2.65 ± 1.58; p<0.001] and sperm progressive motility [Mean±SD (%); A: 33.1 ± 18.0, B: 31.7 ± 17.7, C: 31.4 ± 17.5, D: 28.4 ± 17.5; p<0.001] gradually and significantly decreased with paternal age. The same effect was observed in the patient subpopulation excluding detrimental health and lifestyle conditions [(mL; A: 3.21 ± 1.58, B: 3.05 ± 1.51, C: 2.89 ± 1.59, D: 2.78 ± 1.50; p<0.001); (%; A: 35.85 ± 17.4, B: 33.7 ± 17.4, C: 32.2 ± 17.1, D: 30.3 ± 16.5; p<0.001)]. However, sperm concentration significantly decreased with paternal age in the subpopulation excluding detrimental health and lifestyle conditions [(Mean±SD) million/mL; A: 43.19 ± 41.0, B: 38.8 ± 38.6, C: 38.4 ± 34.6, D: 36.6 ± 33.9; p<0.001], but not in the overall population (million/mL; A: 38.17 ± 40.9, B: 36.7 ± 34.7, C: 35.3 ± 35.1, D: 35. 1± 37.5; p=0.088).
Limitations, reasons for caution: This study is limited by its retrospective nature and by the accuracy of data on health and lifestyle conditions provided by the patients. Differences between age groups not controlled for in the study could also impact the results.
Wider implications of the findings: Ours findings suggest that health and lifestyle conditions may confound the effects of age on semen and sperm quality. Therefore, these data constitute a useful reference for the accurate assessment of the impact of male age on fertility.
Trial registration number: not applicable

P-025 Sperm selection using a modified "swim up" technique in absence of sperm centrifugation improve sperm DNA fragmentation and decreases miscarriage rate

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Study question: Is it useful to avoid sperm centrifugation in laboratory routine work to improve sperm quality and reproductive outcome in Assisted Reproduction Techniques (ART)?
Summary answer: Exclusion of sperm centrifugation for sperm selection using neat sperm samples (IO-lix), increases sperm quality in the collected subpopulation decreasing miscarriage rate after using ICSI.
What is known already: Inclusion of sperm centrifugation in ART is an aggressive intervention for sperm selection with ineludible production iatrogenic damage affecting sperm integrity. The application of IMSI, PICSI or microfluidic devices avoid sperm centrifugation and may improve the quality of the subsample obtained. However, these methodologies may result time consuming, expensive or producing poor results when the quality of the sperm is limited. We have already shown that a modified swim-up avoiding centrifugation (called IO-lix) is a low-cost and efficient alternative to microfluidic devices, recovers 100 times more concentration and reduces sperm DNA fragmentation with no significant differences to other methodologies.
Study design, size, duration: This is a retrospective study from 2018 to 2020 which includes patients with an average of age of 38.2 years using their own oocytes with ICSI as fertilization technique. Two aleatory groups of patients were made: Group 1: 88 cycles with 503 fertilized oocytes and 206 blastocysts were obtained with sperm samples processed by IO-lix and Group 2: 303 cycles, 1451 fertilized oocytes and 591 blastocysts using a standard "swim up" technique to process sperm.
Participants/materials, setting, methods: A total of 391 ICSI cycles were included in this retrospective study. The male factor was similar in both groups and they showed altered SDF previously to the cycle. We compared data of the motility and SDF of sperm samples before and after applying IO-lix and we analyzed by $\chi 2$ contingence test differences on miscarriage rates between groups 1 and 2.
Main results and the role of chance: General sperm parameter changes after IO-lix showed that averaged sperm concentration observed in neat ejaculated samples was 62M/SD=46.4. Values obtained after IO-lix in the same samples were 12.3M/SD8.0. Averaged sperm motility in neat samples was 54%/SD=9.3 and 70.9%/SD=13.2 after IO-lix. Finally, sperm DNA fragmentation in neat samples was 35.8%/SD17.3, while these values decreased to 9.2%/SD=3.9 after IO-lix.

About reproductive outcome results, significant differences were not obtained on the development to blastocyst stage rate comparing both groups ($\chi 2$=0.003; p value = 0.954; Alpha 0.05).

In the case of IO-lix processed samples, the pregnancy rate was 59.42% in Group 1 and 44.72% in Group 2 ($\chi 2$=0.651; p value =0.419; Alpha 0.05).

A total of 9 miscarriages of 41 clinical pregnancies (21.95%) were observed after IO-lix, while this number increases to 59 out of 123 clinical pregnancies, which means the 47.96% of the embryo transfers, when "swim-up" was used. In this case significant differences were obtained ($\chi 2$=3.935; p value = 0.0.047; Alpha 0.05).
Limitations, reasons for caution: Being a pilot study aimed to understand the results of IO-lix in ART, correlations have not been stablished between the levels of sperm improvement after IO-lix and paired results of ART. This study would be necessary, specially to identify the possible origin of miscarriage associated to the male factor.
Wider implications of the findings: Elimination of sperm centrifugation using a combined strategy of gradients and "swim-up" for sperm isolation, reduce miscarriage rate and produce equivalent results of blastocyst development to those obtained with "swim-up". Being a cost-effective and improving laboratory workload, its use for sperm selection is recommended.
Trial registration number: Not applicable

P-026 Seminal plasma exosomes: a promising source of biomarkers for fertility evaluation

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Study question: Do exosomes from seminal plasma have a role in male fertility?
Summary answer: Exosomes isolated from seminal plasma have a pivotal role during spermatogenesis and sperm maturation and may represent eligible biomarkers for male fertility/infertility.
What is known already: During their journey along the male reproductive tract, exosomes contained in seminal fluid are involved in the transfer of several molecules to the maturing sperm. Exosomes are extracellular vesicles (EVs) released by all the cells; they carry a cargo of nucleic acids, proteins and lipids. In the male genital tract, they are released at various levels and their composition differs between men of proven fertility and infertile male patients. Recent studies reported the proteomic profile of exosomes, revealing the presence of several proteins with a well know role in sperm maturation and fertilizing ability acquiring.
Study design, size, duration: This prospective study consisted of 36 Caucasian men; according to seminal parameters (WHO 2010) they were divided in normozoospermic (N; n=12), oligoasthenoteratozoospermic (OAT: n=12) and azoospermic (A; n=12). Semen samples were collected between October 2020 and January 2021 at the Assisted Reproductive Unit, Siena University Hospital (Italy) after institutional ethical approval and signed written consent from all the participants.
Participants/materials, setting, methods: Ejaculated sperm were analyzed according to WHO-2010 criteria and divided into the three groups: N, OAT and A. Exosomes were isolated by an in-house modified ExoGAG®-polymer precipitation-based protocol and characterized for size and ultrastructure by Nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). The exosomal proteins were extracted and analyzed by 2D-electrophoresis and the identified profiles were examined by applying bioinformatic tools. The expression of selected genes was evaluated by digital droplets PCR (ddPCR).
Main results and the role of chance: The present work is readily providing an improvement of the standard ExoGAG® protocol and underlines its advantages over more conventional EVs isolation protocols used to date for recovery from seminal fluid: the number of recovered EVs and their size were finely included in the range of exosomes.

This isolation protocol provides samples suitable for proteomic analyses, representing the first 2D-electrophoresis reference map of exosome-pay loaded proteins in N respect to OAT/A groups and providing an innovative and comprehensive functional overview of its proteins. Moreover, the STRING protein-protein interaction analysis revealed the deregulation of specific pathways (e.g. signaling proteins, chromatin packaging and/or remodeling, protein folding and apoptosis) in A and OAT in comparison with N group.

Gene expression by ddPCR analysis highlighted that most of the analyzed genes are modulated in according to seminal parameters, in particular: *GAPDHS* (*Glyceraldehyde-3-Phosphate Dehydrogenase, Spermatogenic*); *SPAM1* (*Sperm Adhesion Molecule-1*) encoding a members of hyaluronidase family; *ADAM2* (*ADAM Metallopeptidase Domain-2*) that plays an important role in sperm-egg interactions; *CRISP1,2,3* (*Cysteine Rich Secretory Protein 1,2,3*) expressed in the epididymis and secreted into the epididymal lumen; *CLGN* (*Calmegin*) encoding a testis-specific chaperone protein and *PGK2* (*Phosphoglycerate Kinase-2*) expressed in the later stages of spermatogenesis.
Limitations, reasons for caution: This study represents a preliminary experiment. We suggest further comparative studies in larger study cohorts.
Wider implications of the findings: This pilot study, demonstrating the unique proteomic and transcriptomic pattern of exosomes in N/OAT/A groups, supports the importance of exosomes in sperm production and maturation. This methodological set-up is expected to open new ways for advancement in the use of exosomes as fertility biomarkers, making possible personalized approaches in ART.
Trial registration number: Not applicable

P-027 Does air pollution impact on semen parameters? Findings from a real-life cross-sectional study in white-European infertile men

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Study question: We aimed to investigate the association between air pollutants levels and semen parameters in a cohort of white-European men seeking medical attention for couple's infertility.
Summary answer: We found that Pm10, Pm 2.5, and NO2 levels were negatively associated with sperm morphology.
What is known already: Air pollutants levels have been monitored closely for environmental and research issues in industrialized countries.
Study design, size, duration: Data from 156 infertile men consecutively assessed between 01/2019 and 12/2020 were analysed.
Participants/materials, setting, methods: Semen analyses were based on 2010 WHO reference criteria. We analysed the annual average level of the main markers of air pollution (Pm10, Pm2.5, and NO2) between 2014-2018 (Legambiente, 2020, Annual dossier series on air quality in Italy) relative to patients' addresses of the last 5 years. Health-significant comorbidities were scored with the Charlson Comorbidity Index (CCI). Descriptive statistics and linear regression analyses were used to test the association between air pollutants and semen parameters.
Main results and the role of chance: Overall, median (IQR) age and BMI were 37 (33-41) years and 25.1 (23.4-27.3) kg/m2, respectively. A CCI≥1 was found in 12 (7.7%) men, and 47 (30.1%) participants were smokers. As for sperm parameters, semen volume, sperm concentration, sperm progressive motility and normal sperm morphology were 3 (2-4) ml, 18 (5-45.5) x10^6/ml, 32 (22-45) % and 2 (1-3) %, respectively. Pm10 was negatively associated with sperm morphology (ß: -0.580, p=0.001). Furthermore, Pm10 was found to be an independent predictor for sperm morphology worsening (ß: -0.729, p= 0.006) (Fig.1A), after adjusting for age, BMI, CCI and smoking status. Similarly, Pm2.5 levels were negatively associated with sperm morphology (ß: -0.595, p=0.009) (Fig.1B). After adjusting for the same variables, the association between Pm2 and sperm morphology remained significant (ß: -0.724, p= 0.015) (Fig.1C). NO_2 levels were found to be associated with sperm morphology worsening after adjusting for age, BMI, CCI, and smoking status (ß: -0.471, p= 0.006).
Limitations, reasons for caution: It is a retrospective analysis at a single, tertiary-referral academic centre, thus raising the possibility of selection biases. Moreover, markers of air pollutions divided by residence may not completely evaluated the single patient exposure.
Wider implications of the findings: In this cross-sectional study we found that Pm10, Pm 2.5, and NO2 levels were negatively associated with sperm morphology, while they were not consistently associated with an increased risk of other abnormal sperm parameters in infertile men. Further studies are needed to characterize air pollution effects on sperm parameters.
Trial registration number: Not Applicable

P-028 Single centre retrospective analysis of endocrine stimulation therapy prior to microsurgical testicular sperm retrieval (mTESE) in men with hypogonadism and non-obstructive azoospermia (NOA)

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Study question: What is the role of endocrine stimulation therapy prior to mTESE in men with hypogonadism and non obstructive azoospermia (NOA)?
Summary answer: In hypogonadal men there is a positive correlation between change of serum Testosterone (ΔT) before and after stimulation, and a successful mTESE.
What is known already: NOA is the most common cause of azoospermia and it is often associated with hypogonadism and testicular failure. It is common practice for endocrine stimulation therapies such as gonadotropines or selective estrogens receptor modulators to be used prior mTESE; however there is currently paucity of data regarding their efficacy.
Study design, size, duration: Retrospective analysis on infertile men with hypogonadism (defined as T<12nmol/L) and NOA who underwent mTESE with or without prior endocrine stimulation therapy (clomiphene or human chorionic gonadotropin). Retrospective data from 2015-2020, total number of patient: 71; stimulated group (N:40) vs unstimulated group (N:31).
Participants/materials, setting, methods: Retrospective study on infertile men who underwent mTESE with or without prior endocrine stimulation therapy. Hypogonadism was defined as serum testosterone (T) level <12nm/L. We recorded demographic data, cause of testicular failure, previous testosterone therapy, duration and type of endocrine stimulation, pre-and post-stimulation hormone levels(T, FSH, LH), pre-operative hormone levels, successful sperm retrieval rate (at least 1 vial of viable sperm), average Johnsen score and total number of vials of sperm retrieved.
Main results and the role of chance: One-hundred-sixty-eight men underwent mTESE out of which 59 men received endocrine stimulation therapy for NOA between 2015-2020. Among them, we selected men with hypogonadism defined as serum T<12nmol/L which comprised 43% of the entire patient cohort. The hypogonadal group included 71 men, 28/71 had Klinefelter syndrome and 40/71 received endocrine stimulation for 13.9±9.2 months.

Testosterone levels significantly increased after endocrine stimulation (6.3±3.3nm/L vs 11.7±7.4nm/L) with mean change in serum testosterone (ΔT) of 5.7 nm/L (-5.5-23.3, N35). In the stimulated group, pre-operative serum T levels were significantly higher (11.7±7.4 vs 7.8±3.0 p:0.007) as compared to unstimulated men but the success rate of mTESE did not differ significantly (16/40-40%) vs 13/31-42%). Men with Klinefelter syndrome demonstrated significant differences with regards to age, lower T levels, higher FSH and LH levels, lower Johnsen score and success rates compared to other causes of NOA. Comparing men who had successful mTESE vs unsuccessful mTESE - higher T and lower FSH and LH seemed to correlate with successful sperm retrieval. Among men who received endocrine stimulation therapy the ΔT before and after stimulation seemed to correlate with successful sperm retrieval (AUC:0.701, SE:0.089, p:0.043). In the stimulated group a ΔT>3.5nm/L showed a significant association with successful mTESE(p:0.041).
Limitations, reasons for caution: Retrospective study limitations.
Wider implications of the findings: Our study shows a significant improvement of serum T following endocrine stimulation therapy. Overall, in hypogonadal men, the hormonal stimulation seems not to be related to a higher success rate of mTESE but our data do suggest a positive correlation between ΔT before and after stimulation, and a successful mTESE.
Trial registration number: not applicable

P-029 Identification of spermatozoa by unsupervised learning from video data

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Study question: Can artificial intelligence (AI) algorithms identify spermatozoa in a semen sample without using training data annotated by professionals?
Summary answer: Unsupervised AI methods can discriminate the spermatozoon from other cells and debris. These unsupervised methods may have a potential for several applications in reproductive medicine.
What is known already: Identification of individual sperm is essential to assess a given sperm sample's motility behaviour. Existing computer-aided systems need training data based on annotations by professionals, which is resource demanding. On the other hand, data analysed by unsupervised machine learning algorithms can improve supervised algorithms that are more stable for clinical applications. Therefore, unsupervised sperm identification can improve computer-aided sperm analysis systems predicting different aspects of sperm samples. Other possible applications are assessing kinematics and counting of spermatozoa.
Study design, size, duration: Three sperm-like paint images were manipulated using a graphic design tool and used to train our AI system. Two paintings have an ash colour background and randomly distributed white colour circles, and one painting has a predefined pattern of circles. Selected semen sample

videos from a public dataset with videos obtained from 85 participants were used to test our AI system.

Participants/materials, setting, methods: Generative adversarial networks (GANs) have become common AI methods to process data in an unsupervised way. Based on single image frames extracted from videos, a GAN (SinGAN) can be trained to determine and track locations of sperms by translating the real images into localization paintings. The resulting model showed the potential of identifying the presence of sperms without any prior knowledge about data.

Main results and the role of chance: Visual comparisons of localization paintings to real sperm images show that inverse training of SinGANs can track sperms. Converting colour frames into grayscale frames and using grayscale synthetic sperm-like frames showed the best visual quality of generated localization paintings of sperm frames. Feeding real sperm video frames to the SinGAN at different scaling factors, which is defining the resolution of the input image, showed different quality levels of generated sperm localization paintings. A sperm frame given to the algorithm with a scaling factor of one leads to random sperm tracking, while the scales two to four result in more accurate localization maps than scaling levels five to eight. In contrast, scales from six to eight result in an output close to the input frame. The proposed method is robust in terms of the number of spermatozoa, meaning that the detection works well for samples with a low or high sperm count. For visual comparisons, visit our Github page: https://vlbthambawita.github.io/singan-sperm/. The sperm tracking speed of our SinGAN using an NVIDIA 1080 graphic processing unit, is around 17 frames per second, which can be improved by using parallel video processing capabilities. This shows the capability of using this method for real-time analysis.

Limitations, reasons for caution: Unsupervised methods are hard to train, and the results need human verification. The proposed method will need quality control and must be standardized. Unsupervised sperm tracking SinGAN may identify blurry bright spots as non-existing sperm heads which may restrict the use of SinGAN sperm tracking for sperm counting.

Wider implications of the findings: Assessment of semen samples according to the WHO guidelines is subjective and resource-demanding. This unsupervised model might be used to develop new systems for less time-consuming and more accurate evaluation of semen samples. It may also be used for real-time analysis of prepared spermatozoa for use in assisted reproduction technology.

Trial registration number: N/A

P-030 A sperm chromatin damage >15% negatively impacts on the quality of embryos obtained from ovum donation ICSI cycles of unselected couples

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Study question: Is embryo quality downgraded in couples with elevated sperm DNA fragmentation (SDF) in the ejaculated semen of male partner using donated eggs?

Summary answer: The rate of good quality embryos at day 3 and blastocyst-stage is statistically inferior in males with SDF>15% undergoing ICSI cycles with donated oocytes.

What is known already: The effect of a damaged paternal chromatin will be shown from the 8-cell stage of embryo development, a time which the genome of the embryo is transcriptionally active. Fertilization with a spermatozoon with fragmented DNA may impair the quality of the embryos obtained per cycle, and therefore reduce the chances of pregnancy. The use of donated oocytes is an ideal model to evaluate the real effect of SDF on embryo quality by standardizing the female factor. In addition, we have a large cohort of ovum donation cases in our history, which allows a more proper evaluation of the effect.

Study design, size, duration: Retrospective multicentric study including the clinical data of 864 couples of ovum donation program who underwent 1903 ICSI cycles between January 2000 and March 2019. The DNA fragmentation of their ejaculated spermatozoa was measured by TUNEL assay (Terminal deoxynucleotidyl transferase dUTP nick end labeling). Two study groups were created according to the SDF level: ≤15% (low) (n=1626) or >15% (high) (n=277).

Participants/materials, setting, methods: Embryos were evaluated throughout embryonic development according to classical morphological parameters at day 3 (D3), on cleavage-stage, and at day 5 (D5), on blastocyst-stage (trophectoderm (TE) and inner cell mass (ICM)), following ASEBIR guidelines, categorized from A to D. Embryos scored as A and B were considered to be good quality. The proportion of embryos was calculated according to the total number of correctly fertilized oocytes or zygotes. A $p<0.05$ was considered significant.

Main results and the role of chance: A total of 6130 embryos were evaluated. The SDF average of ≤15% group was 5.9% (95%CI 5.7-6.1) and 24.3% (95%CI 23.2-25.3) in the >15% group. The cycle-related characteristics and the seminal parameters were comparable. The proportion of optimal cleavage-stage embryo (number of A+B embryos at D3) per cycle was 21.7% (95%CI 19.0-24.5) (8.1 average cells number, 0.8 embryo fragmentation average, symmetry 1, mononucleated cells) in ≤15% SDF group versus 21.1% (95%CI 13.9-28.3) (8.2 cells number average, 1.3 embryo fragmentation average, symmetry 1, mononucleated cells) ($p<0.001$). The blastocyst-stage arrival rate (number of embryos at D5) per cycle was higher in the >15% SDF group ($p<0.001$), 53.4% (95%CI 48.8-58.1) (TE quality A:20.5%, B:42.5%, C:22.7%, D:14.8%, and the ICM quality A:26.1%, B:52.1%, C:13.2%, D:6.2%) versus 49.9% (95%CI 48.1-51.6) (TE quality A:21.1%, B:42.8%, C:21.85, D:14.1% and ICM A:26.6%, B:55.5%, C:11.1%, D:4.7%) in the low SDF group. The rate of good quality blastocyst (number of quality A+B embryos in D5) per cycle was significantly higher in the couples with low SDF (24.8% (95%CI 23.6-25.9)) than in those with elevated SDF (23.5% (95%CI20.9-26.2)) ($p<0.001$). Accordingly, the A+B blastocyst rate divided by the total number of blastocysts was 59.1% (95%CI 56.7-61.4) versus 55.9% (95%CI 49.9-62.0) ($p<0.001$), respectively.

Limitations, reasons for caution: The main limitation is that retrospective design of the study may not eliminate the potential unaccounted-for bias derived from the clinical practice of multiple centers even though both groups were statistically comparable. Also, the assessment of embryo quality is still remaining highly subjective to embryologists.

Wider implications of the findings: Although the effect size is small, it may be useful in clinical practice when an ICSI cycle yields no good-quality embryos, as one of the underlying causes of that fact. Knowing the SDF level can be a helpful tool in making subsequent clinical decisions aimed at improving outcomes for couples.

Trial registration number: Not applicable

P-031 The effect of ejaculatory abstinence period on embryological and clinical outcomes in ICSI cycles: A retrospective analysis of 3,353 cycles

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Study question: Does ejaculatory abstinence period in male affect embryological and pregnancy outcomes following fresh embryo transfers in ICSI cycles?

Summary answer: Shorter ejaculatory abstinence period is associated with lower triploid zygotes rate per ICSI cycle but it does not affect clinical outcomes after fresh embryo transfers.

What is known already: Lower sperm quality may negatively impact on fertilisation rate and embryo morphokinetic parameters after ICSI and the effect of the ejaculatory abstinence period before semen collection on seminal parameters and sperm quality has been widely reported. However, the impact of ejaculatory abstinence on clinical outcomes is still controversial. WHO (World Health Organization) guideline recommended that abstinence period should be 2-7 days. Even so, there are no larger prospective trials determining the optimal timing for ejaculatory abstinence period for infertile couples.

Study design, size, duration: This is a single center retrospective observational study of 3,353 fresh cycles from January 2017 to December 2020. Semen analysis was done according to the WHO criteria. Exclusion criteria for this study were frozen gametes and cycles with no retrieved oocytes. Primary outcomes were fertilization rate and triploid zygotes rate. Secondary outcomes were blastulation rate, ongoing pregnancy rate and live birth rate per fresh embryo transfer.

Participants/materials, setting, methods: The correlation between ejaculatory abstinence and continuous outcomes was evaluated by Spearman's correlation analysis in order to detect potential non-linear associations. Generalized linear model and logistic regression were used, respectively for continuous and binary outcomes, in order to adjust for confounders such as female age, male age, number of retrieved oocytes, percentage of mature oocytes, infertility causes, seminal volume, sperm concentration and total progressive sperm motility. A p value <0.05 was considered significant.
Main results and the role of chance: The male mean age was 40.3±5.5 and mean duration of abstinence was 2.9±1.7 days. The mean age of female patients was 38.2±4.0. Higher ejaculatory abstinence period was associated with a higher sperm concentration (Spearman $p=3.1x10^{-6}$) but not with a higher total sperm progressive motility. Even so, no significant correlation with EA were observed when considering fertilization rate, blastulation rate, ongoing pregnancy and live birth rate per transfer in analyzed cycles. Triploid zygote rate was positively associated with a higher ejaculatory abstinence period. For the ejaculatory abstinence period of 1 day (n=64), 2 days (n=1523), 3 days (n=1032), 4 days (n=408), 5 days (n=174), 6 days (n=47) and ≥7 days (n=105) the mean triploid rate was 2.4%, 2.4%, 2.5%, 4.1%, 3.6%, 5.4% and 4.3%, respectively (Spearman $p=9x10^{-3}$). Triploid zygote rate was independent of semen volume, concentration and total progressive motility.
Limitations, reasons for caution: This is a large observational study with a retrospective data collection. Despite our methodological approach, the presence of biases related to retrospective design can not be excluded and it may be a reason for caution.
Wider implications of the findings: Our results demonstrate that ejaculatory abstinence period do not affect blastulation, ongoing pregnancy and live birth rates. The current findings discourage an abstinence time longer than 3 days due to its association with a higher abnormal fertilization rate.
Trial registration number: not applicable

P-032 Assessment of embryonic developmental outcome of direct unequal cleavage in patients with non-obstructive azoospermia and/or obstructive azoospermia

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Study question: Does direct unequal cleavage (DC) affect embryonic development after ICSI with testicular sperm (TESE-ICSI) in patients with non-obstructive azoospermia (NOA) and/or obstructive azoospermia (OA)?
Summary answer: The incidence of DC at the first cleavage (DC1) was extremely high and DC1 negatively affected embryonic development in NOA patients.
What is known already: It has been reported that the blastocyst development of embryos with direct cleavage (DC) was significantly lower than that without DC, but the clinical pregnancy rate after blastocyst transfer was not different with or without DC. The incidence of DC has been reported to be significantly higher after ICSI with testicular sperm (TESE-ICSI) than ICSI with ejaculated sperm (Ej), but to our knowledge, there are few reports investigating that the embryos with DC after TESE-ICSI affect embryonic development.
Study design, size, duration: We conducted a retrospective cohort study using time-lapse incubators (Geri, Genea Biomedx, Australia) from September 2018 to November 2020. Of 1033 two-pronuclear (2PN) embryos from TESE-ICSI, 486 and 547 embryos were from OA (35.9±5.5 years) and NOA (33.7±5.2 years), respectively. As an age matched control, we chose 581 embryos from ICSI using Ej (36.5±4.4 years).
Participants/materials, setting, methods: DC embryos were classified as DC1 (DC at first cleavage), DC2 (DC at second cleavage), and non-DC (without DC). The incidences of DC1 or DC2 and blastocyst development rates were compared among OA, NOA and Ej groups. In TESE-ICSI group, we compared blastocyst development rates with or without DC between good and poor quality embryos on day 3. Good quality embryos were defined as 8 cells with G3 or more by the Veeck's classification.
Main results and the role of chance: DC1 incidence was significantly higher in NOA (37.3%) than OA (27.8%) and Ej (22.7%) (P<0.01), whereas DC2 incidence was not statistically different among three groups; NOA (15.7%), OA (15.0%) and Ej (13.4%). Blastocyst development rates in DC1 were 17.8%, 19.5% and 25.8% for NOA, OA and Ej, respectively, which were significantly lower compared to non-DC in corresponding three groups (65.1%, 67.7%, and 68.5%, respectively, P<0.01). In TESE-ICSI group, good-quality embryo rate on day 3 was significantly lower in DC1 (34.5%, P<0.01) than DC2 (60.9%) or non-DC (54.2%). Additionally, blastocyst development rates in DC1 and DC2 were significantly lower than non-DC regardless of embryonic grades on day 3 (35.1%, 51.0%, and 81.6% for good-quality embryos on day 3, 10.1%, 27.0%, and 49.1% for poor-quality embryos on day 3, respectively, P<0.05). When immotile sperm was used for TESE-ICSI, DC1 incidence was 40.0% (6/15), which did not show statistically differences. When performing single frozen-thawed blastocyst transfers, no pregnancies resulted from either DC1 (n=13) or DC2 (n=3) embryos in TESE-ICSI group.
Limitations, reasons for caution: We had a few data about the pregnancy rates after blastocyst transfers with DC, because embryos with DC were seldom transferred due to those lower priority. Although DC might be influenced by the sperm, we did not analyze the incidence of DC by taking the semen factors into account.
Wider implications of the findings: The incidence of DC1 was extremely high and DC1 negatively affected embryonic development in NOA patients. Therefore, it is important to observe embryos using time-lapse incubator in order to recognize embryos with/without pregnancy potential, especially for embryos with DC1 in NOA patients.
Trial registration number: Not applicable

P-033 In vitro protective effect of α-tocopherol and anthocyanin against TiO2-NPs induced genotoxicity on human spermatozoa

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Study question: Do α-tocopherol and anthocyanin counteract human sperm DNA damage provoked by titanium dioxide nanoparticles (TiO2-NPs)? Summary answer: α-tocopherol and anthocyanin are able to counteract TiO_2-NPs genotoxicity on human sperm cells reducing oxidative stress.
What is known already: The environmental release and the extensive use of TiO_2-NPs have been implicated in poor human sperm functionality.TiO_2-NPs is genotoxic on human sperm cells causing a loss of sperm DNA integrity, an increase of apoptotic process and a reduction of genomic stability related to an over production of intracellular ROS.Antioxidants are the substances that can scavenge free radicals. α-tocopherol, present in vegetables, is the most important lipophilic antioxidant involved in restore sperm parameters in several experimental models. Anthocyanin, present in *Aronia melanocarpa*and belonging to the flavonoid family, is able to prevent damage caused by varicocele-induced ROS in rats.
Study design, size, duration: Semen samples from 132 men were obtained by masturbation following 3–5 days sexual abstinence and were examined for sperm concentration, viability, motility and morphology according to WHO 2010. The sperm cells, after purification with 45-90% double density gradient, were exposed *in vitro*to 1 µg/L of TiO_2-NPs, 1 µg/L of TiO_2-NPs whit 1 mg/L of anthocyanin and 1 µg/L of TiO_2-NPs plus 1 mg/L of α-tocopherolfor 15,30,45 and 90 minutes at 37°C.
Participants/materials, setting, methods: Sperm motility and concentration were analyzed with Makler camber while sperm viability and morphology were evaluated by Eosin-Nigrosin Test and by Testsimplets® prestained slides respectively. Antigenotoxicity was evaluated by Comet assay, TUNEL test and RAPD-PCR technique and Genomic Template Stability (GTS,%) calculation. The intracellular ROS level was assessed by DFC Assay. The data were analyzed using ANOVA test by GraphPad Prism 6 and considered significant if p-value ≤ 0.05.
Main results and the role of chance: Sperm analyses showed none statistically significant changes in sperm viability and motility (progressive and non-progressive) for each treatment. Anthocyanin and α-tocopherol counteracted sperm DNA damage induced *in vitro*by TiO_2-NPs neutralizing ROS in a time-dependent way. Comet assay displayed that both antioxidants reduced sperm DNA strand breaks produced by TiO_2-NPs, in particular the damage was no longer statistically significant starting from 30 and 90 minutes of anthocyanin-TiO_2-NPs and -tocopherol-TiO_2-NPs co-exposure respectively. The antioxidant supplementation

induced a statistically decrease of sperm DNA fragmentation provoked by TiO_2-NPs after 45 co-treatment minutes. The RAPD-PCR technique evidenced variations of bands number in the TiO_2-NPs treated sperm compared to the negative control and anthocyanin and α-tocopherol-TiO_2-NPs co-treated samples. Human sperm genomic stability increased after anthocyanin and α-tocopherol TiO_2-NPs co-exposure respect to the TiO_2-NPs single treatment, until it almost reaches the negative control at 90 minutes. Intracellular ROS percentage was significantly lower both in anthocyanin and α-tocopherol TiO_2-NPs co-treated compared to TiO_2-NPs alone starting from 45 minutes.
Limitations, reasons for caution: *In vitro* study. Wider implications of the findings: Our results showed a protective effect of anthocyanin and α-tocopherol on human DNA by neutralizing intracellular ROS induced by TiO_2NPs. We suggest anthocyanin and α-tocopherol as suitable molecules to defend human sperm DNA from oxidative stress, with a potentially role in treatmentof male infertility due to environmental factors.
Trial registration number: None

P-034 Social distancing protocol changes during the COVID-19 pandemic; the effect of at-home semen collection on intrauterine insemination outcomes

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Study question: How have the coronavirus 2019 (COVID-19)-driven changes in semen collection protocols, from on-site to at-home collection, impacted intrauterine insemination (IUI) cycle outcomes?
Summary answer: Our data suggest that at-home semen collection within 2 hours of processing does not negatively impact semen parameters and IUI pregnancy outcomes. What is known already: There are mixed reports regarding the effect of at-home semen collection on IUI outcomes. In a study of 633 cycles, no differences in semen parameters or pregnancy rates were observed between home and clinic collections[1]. Conversely, in a smaller cohort, at-home collection was associated with worse pregnancy outcomes when IUI was coupled with gonadotropin stimulation, but not when coupled with clomiphene[2]. We previously reported no differences in semen parameters and in-vitro fertilization (IVF) embryo transfer outcomes, when cycles using semen collected at-home were compared to cycles with on-site collection[3]. However, such findings cannot necessarily be extended to the IUI setting.
Study design, size, duration: This is a retrospective cohort study of all 529 IUI cycles that took place in 2020 at an academic fertility center. Semen collected at the "clinic" was used for 143 cycles before the COVID-19 pandemic, and "at-home" collected specimens were used for the 386 cycles following the revised semen collection protocol. Participants/materials, setting, methods: Prior to the COVID-19 pandemic, semen was collected at our "clinic" and processed within ~30 minutes. Post-COVID, in order to maintain social distancing, semen was collected "at-home", at an IUI-approved cup, and transported to our center within 2 hours, while maintained to room temperature. Logistic regression models were performed to evaluate the effect of "at-home" collection on achieving pregnancy (positive pregnancy test-PPT) and clinical pregnancy (sonographic confirmation-CP), adjusting for age and anti-Mullerian hormone (AMH).
Main results and the role of chance: The mean age (SD) (years) of the female partner was 35.4 (4.2) vs. 35.4 (4.4) (p=0.978) and of the male partner 36.6 (4.4) vs. 37.1 (p=0.328) for the "clinic" vs. "at-home" groups, respectively. There were no significant differences in day-3 follicle stimulating hormone and AMH. In both groups the most common diagnoses were idiopathic and combined factors infertility (27.3% and 18.9% & 24.1% and 25.1%, respectively for the "clinic" & "at-home" groups, p=0.376). Similarly, there were no differences regarding ovarian stimulation, and gonadotropins were the most common medication used in both groups ("clinic": 44.1% vs. "at-home": 39.4%, p=0.775). Semen analysis parameters (volume, motility, forward progression, total motile count) were comparable between the 2 groups, with the exception of concentration (mil/ml) which was higher with "at-home" collection [66.1 (45.0) vs. 81.1 (63.0), p=0.009].

In unadjusted models, "at-home" collection had no significant effect on the odds for a PPT [OR (95%CI): 0.691 (0.427-1.119), p=0.133] or CP [0.751 (0.447-1.263), p=0.281]. These results persisted even when adjusting for maternal age and AMH: PPT [0.708 (0.435-1.153), p=0.165] and CP [0.773 (0.455-1.312), p=0.340]. When sub-analysis was performed within the different medication groups, the above findings persisted for both gonadotropin and oral medication cycles.
Limitations, reasons for caution: The limitations of the study include its retrospective design and the absence of livebirth data, given the limited follow up period. However, regarding the latter, one can use the ongoing clinical pregnancy rate as an accurate estimate of livebirth.
Wider implications of the findings: At-home semen collection within 2 hours of processing did not negatively impact semen analysis parameters or pregnancy outcomes following IUI. These data constitute an important addition to the current limited literature on the subject and provides an additional level of safety for our patients and staff during the COVID-19 crisis.
Trial registration number: not applicable

P-035 Mathematical model of the signaling cascade during mouse sperm capacitation

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Study question: Modeling the feedback loop controlling sperm capacitation to study the reversibility/irreversibility of the process
Summary answer: We demonstrate the existence of a feedback loop between pH increase and hyperpolarization, inducing bistability and possibly the reversibility of the capacitation process.
What is known already: The process of mammalian capacitation does need an influx of calcium ions through the transmembrane Catsper Channel Complex. This plasmic membrane channel, which is activated by an increase in either the intracellular pH or the membrane potential, is a sperm-specific protein that is localized in the sperm flagella. Two other sperm specific transporters, sNHE and SLO3, found in the flagella, have been shown to control protons influx and transmembrane voltage respectively, and could work together in a feedback loop keeping the sperm in a high pH state with hyperpolarized membrane.
Study design, size, duration: Mathematical model
Participants/materials, setting, methods: Not applicable
Main results and the role of chance: The results given by this minimal theoretical model are robust as the predicted qualitative behavior can accommodate to a wide span of physiological parameters variations.
Limitations, reasons for caution: The model and its parameters are mainly based on in vitro results of the literature, so that the in vivo implications should be inferred carefully.
Wider implications of the findings: The possible reversibility of the capacitation process could have major clinical implications, relevant for the optimization of sperm preparation in assisted reproductive techniques and cryopreservation procedures.
Trial registration number: not applicable

P-036 Investigation of the 2100 MHz electromagnetic field effects on sperm CatSper calcium channel

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Study question: Does electromagnetic field (EMF) effect sperm motility through CatSper calcium channels in rat?
Summary answer: 2100 MHz EMF may reduce sperm motility by acting on CatSper calcium channels.
What is known already: EMF exposure has become a serious concern in infertility patients. The effects of EMF through by using mobile phone and laptop have been explored previously, mostly focusing on sperm motility and DNA fragmentation. EMF activates the voltage gated calcium channels and increases calcium concentration. As a result of the EMA exposure, the sperm motility may increase. However, if this happens while sperms are in non-progressive motile phase in the epididymis, it may result with the depletion of limited energy stores.

Sperms may become immotile and they can't move forward in the progressive motile phase in the female reproductive system.
Study design, size, duration: This basic research study was an in vivo experimental approach involving the use of 50 male rats. Wistar-Albino rats (n=10) weighing ~320 -350g were included in each groups. The duration of EMF exposure was 1 hour per day for 28 days. Amlodipine (1 mg/kg, 28 days) was used as a calcium channel blocker. The experiment was held between July to December in 2020. 20 female rats were recruited for mating test.
Participants/materials, setting, methods: 50 rats were divided into five groups. Group 1; Pure control. Group 2; Sham. Group 3; EMF exposure, Group 4: EMF+Amlodipine, Group 5: Amlodipine positive control. After four weeks of exposure, rats were sacrificed and sperm were collected from cauda epididymis. Sperm motility parameters were analyzed. Intracellular calcium levels were determined with two different method, fluorescence spectrophotometer and laser scanning confocal microscope. Before sacrifice, rats were mated with female rats to evaluate mating ratios.
Main results and the role of chance: The mating score and live birth rates did not vary significantly among the groups (p>0.05). The sperm motility (A+B, 47.62±16.92 versus 34.19±14.62) and intracellular calcium levels (2.46±0.20 versus 1.85±0.18) were significantly decreased in the EMF group (p<0.05). The results of fluorescence spectrophotometer and laser scanning confocal microscopy with fluorescent attachment were consistent with each other. There were no significant differences found among the other groups in terms of investigated parameters. Statistical analysis was performed with Kruskal-Wallis test followed by the Dunn-Bonferroni's test.
Limitations, reasons for caution: The *Catsper* 1, 2, 3, 4 gene expression levels are still under analyses. These gene expression levels will be helpful to understand possible changes of the sperm motility. The determination of other motility related gene expressions may strength the results.
Wider implications of the findings: EMF exposure may have a significant effect on sperm motility parameters. Mobile phones carried very close to the reproductive organs may adversely affect the motility of sperm cells due to its emitted radiation levels
Trial registration number: not applicable

P-037 Influence of sperm selection by microfluidic device on outcomes of in vitro fertilization cycles

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Study question: Does sperm selection by a microfluidic sperm-sorting device improve laboratory outcomes of intracytoplasmic sperm injection cycles?
Summary answer: The ZyMōt™device seems a good alternative to the conventional swim-up technique, possibly increasing progressive sperm motility post processing and blastulation rate.
What is known already: It is well-known that high levels of sperm DNA fragmentation negatively impact not only early embryonic development but also embryo genome activation. Microfluidic technologies for processing sperm samples selection such as the ZyMōt™ device have been developed to sort sperm with lower rates of sperm DNA fragmentation in order to improve *in vitro* fertilization (IVF) outcomes. Moreover, it offers several advantages: working with very small volume samples, high sensitivity and low reaction times, automatic sample treatment and analysis, among others. However, the real benefits on the clinical and laboratory outcomes of IVF cycles is yet to be determined.
Study design, size, duration: We performed a retrospective study including 126 seminal samples processed between May 2019 and December 2020. These samples belong to 63 infertile couples that underwent 2 consecutive IVF cycles using the swim-up technique in the first cycle (n=63) and the ZyMōt™ device in the second (n=63). The oocytes of the female partners were recovered and inseminated, and the embryonic culture was carried out until the blastocyst stage, when the quality of these embryos was analyzed. Participants/materials, setting, methods: A total of 118 seminal samples from 59 couples were included. Four couples were excluded due to insufficient seminal volume / concentration (2) or absence of mature oocytes recovered (2). Laboratory outcomes were evaluated including semen parameters post processing, as well as fertilization, cleavage, blastulation (blastocyst among cleavage stage) and top-quality blastocyst rates. Continuous variables were analyzed by paired Student's *t*-test or Wilcoxon signed-rank test, as appropriate. Differences were considered significant when *p-value* < 0.05.
Main results and the role of chance: We did not find any difference in the percentage of non-progressive sperm motility [0 (0-33%) *vs.* 0 (0-10%); *p*=0.063] and immotile sperm [0 (0-70%) *vs.* 0 (0-80%); *p*=0.095] post processing when comparing the swim-up technique with the ZyMōtTM device. Likewise, no significant differences were detected regarding fertilization [83% (0-100%) *vs.* 89% (0-100%); *p*=0.104] and cleavage [100% (0-100%) *vs.* 100% (80-100%); *p*=0.217] rates in both groups. Nevertheless, there was a significant difference in the percentage of progressive sperm motility [100% (20-100%) *vs.* 100% (10-100%); *p*=0.012] post processing, as well as blastulation [37% ± 0.32 *vs.* 50% ± 0.3; *p*=0.031] and top-quality blastocyst [13.5% (0-100%) *vs.* 33% (0-100%); *p*=0.009] rates, favoring the ZyMōt™ device.
Limitations, reasons for caution: The retrospective design and the small sample size make the study prone to bias. Furthermore, we do not have data on sperm DNA fragmentation pre and post processing. Clinical outcomes were not evaluated due to insufficient statistical power, since embryo transfer has not yet been accomplished in several patients.
Wider implications of the findings: Besides the ZyMōt™ device has been indicated as a new tool to potentially improve laboratory and clinical IVF outcomes, it offers the advantage of being safe, fast, easy-to-use and less influenced by human errors. Further well-designed prospective studies are needed to prove its cost-effectiveness.
Trial registration number: not applicable

P-038 Clinical outcomes of 77 testicular sperm extraction treatment cycles in non-mosaic Klinefelter syndrome patients

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Study question: What are the clinical results of patients with azoospermia and nonmosaic Klinefelter syndrome, using fresh and cryopreserved sperm?
Summary answer: The results showed a recovery rate of testicular sperm in the order of 40% and a life newborn rate of 52% when using fresh sperm
What is known already: In Klinefelter syndrome (KS), the rates of successful testicular sperm retrieval were shown to be similar either using conventional TESE or micro-TESE (Corona et al., 2017), which highlights that the variability observed between studies is due to differences in patient characteristics. There are a few works with a large number of KS patients elucidating the clinical outcomes using fresh and cryopreserved testicular sperm. However, these studies revealed contradictory outcomes, either revealing better (Greco et al., 2013; Vicdan et al., 2016) or worst (Madureira et al., 2014) results with cryopreserved testicular sperm, or finding no differences (Chen et al, 2019).
Study design, size, duration: This study includes all patients up to 2019 presenting azoospermia due to non-mosaic Klinefelter syndrome (n=76) that went for infertility consultations in a private fertility clinic. Patients were evaluated by the same Urologist. The genetic analysis of the patients was performed at an academic institution. At examination patients did not refer other complaints besides infertility, and referred to have not received any hormone replacement therapy in the past.
Participants/materials, setting, methods: The 76 azoospermic patients with non-mosaic Klinefelter syndrome (KS) were treated by testicular sperm extraction (TESE) followed by intracytoplasmic sperm injection (ICSI), using fresh and cryopreserved testicular sperm. Most patients used fresh testicular sperm, where others preferred to postpone ICSI treatment cycles and used cryopreserved testicular sperm. Aneuploidy screening in children was performed by prenatal diagnosis and MLPA (Multiplex ligation-dependent probe amplification). Full embryological, clinical and newborn outcomes are provided.
Main results and the role of chance: Of the 76 patients with non-mosaic Klinefelter syndrome, one repeated the testicular sperm extraction (TESE) procedure. Testicular sperm were recovered in 31/77 (40.3%) of the cases. Comparisons between the 31 cases with successful sperm recover (group-1) and

the 46 cases without a successful TESE (group-2) revealed no significant differences regarding age, time of infertility, testicular volume, serum levels of FSH, LH and testosterone, total number of testicular fragments analyzed, and time of search in samples. The mean male age was 34 years. In most of the cases, the testicular volume was reduced (96.1%), the levels of FSH (98.3%) and LH (94.1%) were increased, and the levels of testosterone were normal (77.6%). There were 25 intracytoplasmic sperm injection (ICSI) treatment cycles using fresh testicular sperm and 22 ICSI treatment cycles using frozen testicular sperm. The rates of fertilization (63.5% fresh sperm vs 41.6% frozen sperm), implantation (37% fresh sperm vs 13.2% frozen sperm), clinical pregnancy (60.9% fresh sperm vs 19% frozen sperm), live birth delivery (52.2% fresh sperm vs 19% frozen sperm) and newborn (65.2% fresh sperm vs 23.8% frozen sperm) were higher in the group using fresh testicular sperm. Chromosome analysis of the 21 newborn was normal.
Limitations, reasons for caution: Although presenting a high number of cases with azoospermic non-mosaic Klinefelter syndrome treated with testicular sperm extraction and intracytoplasmic sperm injection, future studies are needed with a higher number of cycles using frozen testicular sperm, in order to confirm or rebut that the freezing methodology affects negatively the clinical outcomes.
Wider implications of the findings: Data adds further information regarding testicular sperm retrieval rates and use of fresh or frozen testicular sperm in Klinefelter syndrome (KS) patients. High newborn rates were obtained only with fresh testicular sperm. Results also reassure KS patients about the safety relative to any abnormal chromosomal transmission to the born children.
Trial registration number: Not Applicable

P-039 Sperm chromatin integrity in relation to clinical pregnancy rate in an egg donation program

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Study question: Is DNA Fragmentation Index (DFI) determinant in clinical pregnancy rate (CPR) after ICSI?
Summary answer: DFI is determinant in CPR after ICSI
What is known already: Male fertility evaluation often leaves the clinician in uncertainty. Semen analysis is a basic examination, but insufficient. Sperm DNA is in a compact state, and its integrity is observed to be related to reproductive capacity
Study design, size, duration: A retrospective, single center study, in 65 couples underwent egg donation, ICSI and blastocyst transfer, evaluating DFI and its effect on CPR. This study was carried out since September 2017 to March 2020
Participants/materials, setting, methods: DFI was evaluated using Sperm Chromatin Dispersion test (SCD) and considering 20% or below as normal. We performed ICSI in donated eggs, cultured until blastocyst stage and transferred 1 to 3 embryos. CPR was defined as the number of patient with fetal heart beats presents in relation with the number of patients with embryo transfer. We determined CPR in both groups with normal and abnormal DFI. Fisher exact test was used to analyze the differences.
Main results and the role of chance: From these 65 couples, in 29 male partners had normal DFI and 36 abnormal. CPR was 68.97% in the first group and 41.67% in the second (p = 0.0448)
Limitations, reasons for caution: The small number of patients is a limitation
Wider implications of the findings: The results permit us to know more male patients, to consider DFI as an important variable and to prepare better our patients for procedures.
Trial registration number: 00012

P-040 Correlation between DNA fragmentation index and big halo pattern with sperm decondensation index and semen sample parameters

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Study question: Does sperm DNA integrity evaluated by DNA fragmentation index (DFI) and big halo pattern correlate with sperm decondensation index (SDI) and semen sample parameters?
Summary answer: DFI correlates with SDI and semen sample parameters in a stronger way than the big halo pattern
What is known already: The sperm chromatin dispersion test evaluates DNA integrity by measuring the susceptibility of sperm DNA containing breaks to denature when treated by an acid solution. Spermatozoa with intact DNA produce big or medium size halos of dispersed DNA loops, whereas small halos or no halos indicate fragmented DNA. The DFI calculates the proportion of spermatozoa with fragmented DNA. Data have been published documenting the negative effect of sperm DFI on embryo viability, suggesting that its evaluation could contribute to the prediction of the male reproductive potential
Study design, size, duration: A prospective study between 2011 to 2019 included 300 patients attending our clinic for fertility treatment. All sperm samples were analyzed according to WHO criteria, and the results from the DNA integrity analysis were related to the semen sample indices
Participants/materials, setting, methods: Of the 300 males included in the study, 118 were normozoospermic, 16 were oligozoospermic (O), 63 were asthenozoospermic (A), 9 were teratozoospermic, 7 were AT, 51 were OA, 5 were OT, and 31 OAT. The DNA integrity was assessed by the Halosperm test, and DNA decondensation by the aniline blue assay. A big halo was defined as a dispersion greater or equal to the length of the minor diameter of the core
Main results and the role of chance: DFI showed negative correlations with progressive motility (r= -0.532, p= 2.816 E-23), total motility (r= -0.598, p= 1.688 E-30) and morphology (r= -0.338, p= 2.954 E-9). Accordingly, when compared with normozoospermic, DFI was significantly higher in A and T samples (29.5 ±12.0 and 36.5±4.8 respectively, p<0.002) with the highest levels found in samples with combined defects (45.2±12.5 in AT, p<0.002; 51.3±17.2 in OAT, p<0.002). DFI also showed a negative correlation with the big halo pattern (r= -0.656, p= 2.934 E-38) and a positive correlation with the SDI (r= 0.429, p= 7.314 E-15). For the big halo, negative correlations were found with progressive motility (r= 0.429, p= 7.314 E-15) and morphology (r= 0.407, p= 4.077 E-13) resulting in a lower incidence in T samples (27.0±9.6, p<0.002) that was especially relevant in AT (18.3±14.5, p<0.002), OT (33.0±10.2, p<0.02) and OAT samples (20.6±15.8, p<0.002). SDI presented a negative correlation with total motility (r= -0.403, p= 3.849 E-13) and was fond to be increased in A samples (32.4±11.8, p<0.002) as well as in samples with double defects (38.9±19.2 in AT samples and 38.8±15.9 in OA samples, p<0.002) and triple defects (42.6±16.8 in OAT, p<0.002)
Limitations, reasons for caution: The study did not evaluate the lifestyle and reproductive history of the patients
Wider implications of the findings: Although the effects of sperm DNA damage on reproductive outcomes are still unclear, the correlation between sperm DNA fragmentation, semen parameters and reproductive potential is emerging. DFI, big halo and SDI could contribute to the diagnosis of male infertility especially in categories of patients with poor prognosis of pregnancy.
Trial registration number: Not applicable

P-041 Reproductive concerns and sexual health in men with newly diagnosed testicular cancer prior to orchiectomy: preliminary results from an on-going study

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Study question: What is the prevalence of reproductive concerns among patients with newly diagnosed testicular cancer (TC), and how do they rate their sexual health (SH).
Summary answer: Of 20 patients, 75% (n = 15) were moderate to highly concerned about decreased reproductive functioning. Twenty-four percent exhibited overall low SH.

What is known already: Currently, only little is known about reproductive concerns among TC patients. Furthermore, these concerns have not been investigated in a TC population prior to orchiectomy. One study among post-surgery TC patients two years after diagnosis, reported that 28 % had high degree of reproductive concern. The literature indicates that TC patients' sexual health is negatively affected due to altered body image and sexual dysfunction. However, studies regarding sexual health have primarily been performed on long term survivors of TC. Thus, little is known about SH in this population prior to treatment.
Study design, size, duration: The present cross-sectional study included patients from the fertility clinic in Horsens, Denmark. Patients were approached at their pre-scheduled appointment for cryopreservation of semen prior to orchiectomy. Enrolment started March, 2019 and is still on-going. Preliminary data is included from 21 enrolled patients.
Participants/materials, setting, methods: Patients newly diagnosed with TC, who were referred to the fertility clinic for semen cryopreservation prior to orchiectomy and other treatment modalities were invited. The patients responded to a questionnaire package of which reproductive concerns were assessed with seven questions with Likert scale response options ranging from 0 (not concerned) to 5 (highly concerned). SH was assessed with the validated 22-item questionnaire European Organization for Research and Treatment of Cancer Sexual health questionnaire (EORTC-SHQ).
Main results and the role of chance: A total of 37 patients met the inclusion criteria and of these 21 were enrolled in the study. Due to technical issues, only 20 out of 21 patients completed the full questionnaire package. Patients were asked about concerns regarding the ability to father children. Nine patients (45%) were moderately concerned, and six patients (30%) were highly concerned. When asked about their concerns of not being able to father children without fertility treatment, the answers were mostly unaffected with eight patients (40%) being moderately concerned, and seven patients (35%) highly concerned.

Four patients (20%) were highly concerned that decreased semen quality would affect future or present relationships. Patients were also asked if they felt sufficiently informed regarding the chance of fathering children without help from a fertility clinic following cancer treatment. Three patients (15%) reported that they were insufficiently informed, while four patients (20%) responded only to a little extend. Five patients (23,8%) scored ≤ 50 on the EORTC SHQ indicating that they had low SH. Eleven patients (52,3 %) felt less masculine due to their disease. Furthermore, one patient (4,8 %) scored ≤ 50 on the symptomatic scale, indicating that he had symptomatic sexual problems as fatigue and sexual pain.
Limitations, reasons for caution: The relatively low participant number is a limitation, making the results less generalizable. Furthermore, there is a risk of selection bias due to the moderate inclusion rate. Also, the questionnaire examining fertility related concerns were non validated, and focused mainly on the fertility-related aspects of reproductive concerns.
Wider implications of the findings: A considerable number of patients with newly diagnosed TC show substantial reproductive concerns as well as lowered sexual health. These worries could possibly be alleviated by more sufficient information from the health professionals already in the beginning of the treatment phase, reducing further emotional distress during the remaining treatment period.
Trial registration number: ClinicalTrials.gov ID:NCT03880994

P-042 Impact of semen parameters, sperm DNA fragmentation and sperm aneuploidy in male infertility

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Study question: Should sperm aneuploidies and sperm DNA fragmentation (sDNAfrag) be included as valid tests in the routine investigation of male infertility?
Summary answer: Sperm DNA fragmentation was associated with male age, oligozoospermia (OZ), oligoteratozoospermia (OT), astenoteratozoospermia (AT) and oligoastenoteratozoospermia (OAT). Sperm aneuploidies were associated with OT and OAT.
What is known already: Semen parameters assist male infertility diagnosis and treatment, but sDNAfrag and aneuploidy analysis could add useful information, as abnormal values compromise fertility. To include these tests in the routine diagnosis it should be determined if behave as informative parameter and add information regarding the fertility status. For that, further studies comparing these tests to semen parameters are needed, since previous results are not consensual. Additionally, standardization of a sDNAfrag cut-off is needed, as different sample sizes and techniques originate distinct results. Also, until a standardization of the protocol is missing, a cut-off value should be defined for each laboratory.
Study design, size, duration: A retrospective and prospective investigation was performed, within a 12 years period (April 2007-December 2019). A total of 835 infertile males with a normal karyotype (46,XY) were included. Karyotyping and evaluation of sDNAfrag and sperm aneuploidies were made at a public Genetic unit. All normozoospermic (NZ) patients with a born child and patients whose infertility treatments were done due to female factors were selected from our database and used as controls (60 individuals).
Participants/materials, setting, methods: Semen analysis followed WHO-2010 guidelines. sDNAfrag was evaluated using the TUNEL assay. Sperm aneuploidies were detected using FISH (chromosomes 13, 18, 21, X, Y). Several tests were applied: correlations for linear associations between numerical variables, ANOVA for comparisons between means, Dunn-test for post-hoc comparisons. To determine the sDNAfrag cut-off value, the area under the ROC curve, sensitivity and specificity, were calculated, with the Youden-Index used to find a threshold that maximizes both sensitivity and specificity.
Main results and the role of chance: Regarding male age, it was observed a positive correlation with sperm concentration, a negative correlation with sperm vitality (VT) and hypoosmolality, and a positive correlation with sDNAfrag. Regarding sDNAfrag, it was observed negative correlation with sperm concentration, total progressive motility (TPM), morphology, VT and hypoosmolality. Regarding sperm aneuploidies, both total sperm aneuploidy and total sperm disomy exhibited a negative association with sperm concentration, TPM and morphology. It was also investigated whose groups of individuals could be indicated for sDNAfrag or sperm aneuploidy testing. The NZ group evidenced significant lower sDNAfrag, total sperm aneuploidy and total sperm disomy in relation to the non-NZ group. In the NZ group, sDNAfrag was significantly lower in relation to the OZ, OT, AT and OAT groups. The NZ group presented significant lower percentages of sperm aneuploidy in relation to the OT and OAT groups, and significant lower percentages of sperm disomy in relation to the OAT group. Additionally, sDNAfrag was positively correlated with total sperm aneuploidy and total sperm disomy. From the present large population, ROC curve analysis allowed estimating a cut-off value of 18.8% for the TUNEL-assay (sDNAfrag), with 0.658 of area under the curve, 53.9% sensitivity and 76.7% specificity.
Limitations, reasons for caution: Although presenting a high number of cases and strict controls, the present study was unable to include as controls healthy men with proven fertility. Additionally, the present study did not take into account life-style factors and male associated pathologies besides infertility

Wider implications of the findings: Semen parameters were shown to be negatively correlated with sDNAfrag and sperm aneuploidies. As sDNAfrag testing and sperm aneuploidy testing were associated with semen abnormalities and male age, it is suggested their inclusion in the routine evaluation of infertile men, thus adding important complementary information about the fertility status.
Trial registration number: Not Appliable

P-043 Six years' retrospective statistical study (2013 – 2018) investigating the impact of Sperm DNA fragmentation on sperm analysis parameters

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Study question: What is the relationship between Sperm DNA fragmentation (SDF) levels and sperm analysis (*Spermocytogramme*) parameters results?
Summary answer: SDF level of patients with pathological *spermocytogramme* presents negative correlations to total spermatozoa mobility, vitality and concentration, and positive correlation to sperm morphology defects.
What is known already: The relationship between SDF and Sperm analysis parameters and especially sperm morphology needs to be more studied since few studies over the last years were focused on this relationship. However, abnormalities in these two parameters are considered as the most important biological indicators of male infertility. The pathogenesis of Teratozoospermia (<4% morphologically normal sperm cells according to WHO 2010) is continuously increasing over the last decade according to several studies. In addition, SDF is also increasing over the years because of several factors such as pollution, stress and lifestyle changing.
Study design, size, duration: Retrospective study including 331 infertile patients undergoing SDF-index testing with *Spermocytogramme* from January 2013 – December 2018. Patients divided into two groups: 143 patients with normal-*Spermocytogramme* and 188 patients with pathological-*Spermocytogramme*. Each group includes patients with abnormal SDF levels (>30%). Statistical analyzes were performed using SPSS22.0 for Windows-software. Kolmogorov–Smirnov-test for normality analysis and comparisons by Student-t-test or Mann–Whitney U-test, as appropriate. Pearson/Spearman' tests for correlations were used as appropriate, *P-value*<0.05 was considered as significant.
Participants/materials, setting, methods: 143 patients with normal *Spermocytogramme* (2.8% abnormal-SDF) vs 188 patients with pathological *Spermocytogramme* (10.6% abnormal-SDF). WHO-2010 instructions for sperm-analysis were used through Makler®-counting-chamber (Sefi-Medical Instrument Ltd) for sperm-concentration and motility-determination using Sperm-class-analyzer-software (CASA-system (Microptic®)) to detect sperm abnormalities. Normozoospermia was determined when sperm progressive-motility is ≥32%, sperm-concentration ≥15x10^6/mL, and sperm-morphology ≥4%. "Diff-Quick" staining-method for the coloration of the fixed-sperm-slides was used for Sperm-morphology analysis. GoldCyto Sperm®Kit (Goldcyto Biotech corp.) was used to analyze SDF.
Main results and the role of chance: SDF is significantly higher in pathological *spermocytogramme*' patients than in normal *spermocytogramme*' patients (17.02 ± 11.88 vs 12.16 ± 9.58 respectively). In patients with pathological *spermocytogramme*, SDF is negatively correlated to Progressive sperm motility (r= -0.137; p=0.042), Total sperm motility (r=-0.153; p=0.036), vitality (r=-0.140; p=0.048) and concentration (r=-0.195; p=0.007). In the other hand, SDF presented positive correlation with teratozoospermia and especially with sperm midpiece defects (r=0.171; p=0.02). However, SDF did not present any correlation with age, testosterone levels and total ejaculated sperm volume. However the latter was positively correlated to spermatozoa midpiece and head defects (r=0.156; p=0.034; r=0.203; p=0.006, respectively). These results are in accordance with García-Ferreyra *et al.* (2014) who found that men with abnormal spermatozoa morphology showed high levels of DNA fragmentation, Sá *et al.* (2015) who confirmed that semen with lower concentration, motility and morphology have higher levels of SDF and showed that sperm head staining patterns are correlated with the degree of SDF. In addition, recently the study of Jakubik-Uljaszstudy *et al.* (2020) could confirms our results when it concluded that detailed sperm structural defects coexist with abnormal nuclear sperm DNA dispersion and that men with teratozoospermia may have a higher risk for sperm DNA damage.
Limitations, reasons for caution: Our study is a retrospective statistical investigation that included patients attending to the laboratory for fertility diagnosis after a period of infertility. Meta-analyzes studies in addition to more prospective-randomized-controlled-trials with couples undergoing assisted-reproductive-treatments and in comparison with fertile men are needed to confirm the relationship between SDF and *spermocytogramme* defects.
Wider implications of the findings: These results should interest andrologists, reproductive science fundamentalists and embryologists who want to improve the investigations on the origin of infertility especially when it comes from male side.
Trial registration number: not applicable

P-044 A non-classical splice site variant in ANOS1 gene leading to Kallmann syndrome

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Study question: Genetic risk of the non-classical splice site variant in *ANOS1* gene
Summary answer: A non-classical *ANOS1* splice site variant, c.1062+4T>C, causes Kallmann syndrome.
What is known already: Genetic abnormalities play a key role in the development of Kallmann syndrome. Although an overwhelming majority of missense and nonsense mutations occur in the exons of a gene, intron mutations can also be pathogenic.
Study design, size, duration: The research object is a family. Eight patients of the family were recruited in this study, three of them were diagnosed with Kallmann syndrome.
Participants/materials, setting, methods: Genomic DNA was extracted from peripheral blood and whole-exome sequencing (WES) was performed to identify the genetic abnormalities. PCR was performed to verify the WES results. The functional splicing reporter mini gene assay was performed to assess the impact of sequence variants on splicing.
Main results and the role of chance: The proband and other two patients exhibited the typical clinical features of KS. A non-classical splice site variant, c.1062+4T>C in *ANOS1* gene was identified, whereas the other unaffected family members did not have this mutation. This mutation caused the disappearance of the splicing site of intron 7 and the splicing position became the 156th base of exon 7, which caused a frame-shift mutation, leading to a premature termination of translation.
Limitations, reasons for caution: Since the ANOS1 gene is almost not expressed in the blood, in order to uncover the effect of this splice site variant of ANOS1, we carried out a functional splicing reporter mini gene assay in the mini gene vector pEGFP-N1.
Wider implications of the findings: This study shows that mutations in non-classical splicing regions are also pathogenic. Therefore, it is recommended that the detection and analysis of this gene should pay attention to the non-classical splice site variant.
Trial registration number: not applicable

P-045 A retrospective study on Hyaluronic acid binding sperm selection in ICSI cycles

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Study question: Does Hyaluronic acid(HA) binding sperm selection prior ICSI produce better outcome compare to PVP?
Summary answer: No significant difference in fertilization rate, blastocyst utilization rate, pregnancy rate and miscarriage rate in HA vs PVP group.
What is known already: HA is natural, non-sulphated glycosaminoglycan secretion that abundantly found in the cervical mucus and the cumulus oophorus complex (COC). In in-vivo, HA binding sites on the sperm plasma membrane indicate sperm maturity, mature sperm reaching HA-coated COC can bind and

digest HA and subsequent hyperactivation further facilitate fertilization. In IVF, the use of HA tries to mimic in-vivo to select mature sperm with high DNA integrity prior to ICSI. In HA, movement of mature sperm is 'slowed' thus allow the selection of sperm to be used in ICSI. Sperm immaturity is known to associated with aneuploidy incidence in the embryos.

Study design, size, duration: ICSI cycle using HA (N=83) was adapted from January to December 2020 while ICSI cycle using PVP (N=133) was adapted from January 2018 to December 2019. Mean age of patient were 35.64±4.33 vs 34.15±4.75 for HA vs PVP group respectively. Fertilization rate, blastocyst utilization rate, pregnancy rate and miscarriage rate were recorded. This study included all ICSI cycle and both frozen and fresh embryo transfer data. Surgical retrieved sperm was excluded from this study.

Participants/materials, setting, methods: A 1.5ul of treated spermatozoa suspension was connected with a pipette tip to a 5ul droplet of fresh holding medium (Cook Gamete Buffer). Simultaneously, a 5ul droplet of HA medium (Origio SpermSlow) was connected to the 5ulL droplet of holding medium in a ICSI dish (Sparmed Oosafe) covered with oil (Vitrolife Ovoil). Sperm which were 'slowed' in the Sperm slow droplets with normal morphology according to WHO 2010 guideline were selected for ICSI at 400X.

Main results and the role of chance: The fertilization rate of the HA vs PVP- binding sperm are 68.6% vs 66.2%. As for the blastocyst utilisation rate is 61.6% vs 73.22% for HA vs PVP- binding sperm group. Pregnancy and miscarriage rate for HA vs PVP are 42.3 % vs 51.5 % and 19.4% vs 26.2% respectively.

There was no significant difference in the fertilization rates, blastocysts utilisation rate, pregnancy rate and miscarriage rate between the HA vs PVP- binding sperm groups (P<0.05). However, a trend of higher pregnancy (51.5% vs 42.3%; P=0.279) and miscarriage rate were observed in the PVP group (26 % vs 19 %; P = 0.545) as compared to the HA group, but the difference was not statistically significant. The reason behind this might be the HA assist to select the mature sperm with higher DNA integrity and low frequency of chromosomal aneuploidies which contribute to the lower miscarriage rate in the study.

Limitations, reasons for caution: This is a retrospective study on HA binding sperm selection vs PVP prior to ICSI.

Further research which includes a large number of RCT sample size should be warranted.

Wider implications of the findings: The HA- sperm binding selection ICSI might only be beneficial to certain group of patients (high- DNA fragmentation sperm). A larger RCT study may be necessary to establish a relationship between HA-sperm binding selection vs aneuploidy rate via PGT analysis.

Trial registration number: NOT APPLICABLE

P-046 Application of hyperspectroscopy as a new sperm diagnostic and selection technique for ICSI based on hyperspectral signature of single spermatozoa – a proof of concept

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Study question: Is hyperspectral imaging of individual sperm cells an appropriate candidate technique with potential to identify molecular characteristics of single spermatozoa prior to microinjection?

Summary answer: Preliminary tests have allowed us to obtain the images, set up the methodology and the correct segmentation of each sperm into its signature spectra.

What is known already: Although image techniques such as fluorescence microscopy and Raman spectroscopy have been used to identify biomarkers on sperm cells, their translation to the fertility clinic is highly complex and unfeasible. Hyperspectroscopy has not yet been introduced in the reproductive field, but it has been successfully used in other medical disciplines to differentiate cell types or pathological versus healthy tissue. It relies on the combination of information from optical images and electromagnetic spectra produced by cells at different wavelengths. Therefore, it is an interesting candidate for objective non-destructive sperm selection before ICSI according to the presence of specific biomarkers.

Study design, size, duration: Pilot, prospective, observational study in the IVIRMA Valencia fertility clinic, designed as a proof of concept. Discarded semen samples were used for the technical setup and the preliminary tests of image acquisition. Hyperspectral images were obtained of 12 spermatozoa: two sperm cells were imaged 10 times each, while the other 10 sperm were imaged one time each.

Participants/materials, setting, methods: Samples were prepared on glass-bottom ICSI plates in PVP droplets according to routine clinical practice. Images were obtained using the camera HinaLea 4200M coupled to the Olympus IX73 inverted microscope. Hypercubes were extracted using MATLAB 2016a, and the most informative wavelength was chosen. Matrox Copilot software was used to process images and select the regions of interest (ROI) equivalent to one sperm. MATLAB was used to obtain the information for the pixels within each ROI.

Main results and the role of chance: Hypercube size for each image was 608x968x299 pixels. Each hypercube obtained provided information for 299 different wavelengths, from 402.8 to 998.3 nm. Once the hypercubes were obtained from each image, the spectra at 520 nm wavelength was selected as the one at which the pixel provided the most information. The background of each uploaded image was successfully removed by creating a mask, which was then used to extract and characterize all the spectra from each pixel of each sperm image. The result of this proof of concept, though measure qualitatively, is that the information obtained from hyperspectral imaging of individual sperm is highly reproducible and successful in showing overlapping spectra resulting from 10 different images of the same specimen. Spectra resulting from images of different sperm cells showed tangible differences on their composition demonstrating unique biochemical features of each. The next step will be to quantitatively measure the reproducibility and sensitivity of the technique in a larger sample, to then study the potential of single sperm hyperspectral imaging as a sperm selection tool for ICSI.

Limitations, reasons for caution: These are preliminary results obtained from the development of an appropriate methodology to adapt the acquisition of hyperspectral images in the clinic's IVF laboratory. Thus, the group will continue to evaluate the reproducibility, sensitivity, and informative capability of the technique before contemplating its use on samples destined to perform cycles.

Wider implications of the findings: Having developed a laboratory and image processing methodology that allows us to successfully segment each obtained image according to the hyperspectral information of every pixel of each individual sperm, we could move forward in testing the potential of these hyperspectral signatures to identify sperm markers related to successful reproductive outcomes.

Trial registration number: NOT APPLICABLE

P-047 Knowledge, professional attitudes and training of health professionals on male contraceptive methods

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Study question: Among health professionals involved in contraceptive prescribing, what are the knowledge, professional attitudes and training on male contraceptive methods?

Summary answer: The health professionals involved in prescribing contraception are not sufficiently trained in male contraception and almost all of them want more.

What is known already: The most recent large-scale studies show that 70% of couple contraception is provided by women and that the majority of men and women would be willing to adopt male contraception as couple contraception. The medicalization of contraception places the medical

profession at the forefront of the acceptability of and information regarding a contraceptive method. However, only one study have evaluated health professionals' knowledge of the various methods of male contraception (MC), including male hormonal contraception (MHC) and male thermal contraception (MTC).

Study design, size, duration: Between April 2020 and June 2020, we carried out a descriptive prospective multicentre study in a medical population of 2243 prescribers of couple contraception in France.

Participants/materials, setting, methods: The participants were obstetrician-gynaecologists, medical gynaecologists, general practitioners or midwives. They completed a three-part numerical questionnaire, including i) sociodemographic characteristics and personal experiences with contraception, ii) knowledge and professional attitudes about male contraception and iii) training on male contraception.

Main results and the role of chance: The overall participation rate was 19% (340/2243). Condoms and withdrawal were known by 98% and 89% of the population, respectively. Vasectomy was known by 75% of the population and significantly better known by obstetrician-gynaecologists than by medical gynaecologists and general practitioners (p=0.026). Male hormonal contraception (MHC) and male thermal contraception (MTC) were known by 10% and 23% of the population, respectively, and were significantly better known by medical gynaecologists and general practitioners than by other specialties (p<0.001). More than half (55%) of the population never or infrequently offered MC during a couple's contraceptive request consultation. Female practitioners offered MC significantly more often than male practitioners (48% vs. 26%; p=0.033). Only 14% of the population had ever participated in training on MC, 96% wished to be better trained on MC, and 86% expressed a willingness to participate in such a training.

Limitations, reasons for caution: The population was mainly representative of medical health practitioners of southeastern France. There was an over-representation of women in all medical specialties, except for midwives.

Wider implications of the findings: Our study shows that health professionals involved in contraception have limited knowledge about MC and are eager to have more information about it. To advance the acceptability and dissemination of such contraceptive methods, it seems imperative to provide health professionals with an adapted training program on male contraception.

Trial registration number: 2020-01-23-03

P-048 Effects of bisphenol S and bisphenol F on human spermatozoa: an in vitro study

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Study question: Are plasticizers bisphenol S (BPS) and bisphenol F (BPF) safer alternatives to bisphenol A (BPA) for human sperm function?

Summary answer: Unlike BPA, the analogues BPS and BPF do not significantly affect human sperm mitochondrial functions, motility and viability

What is known already: The widespread distribution of BPA, along with its reputation to be an endocrine disruptor has generated concerns about possible adverse effects for human health, thus prompting the European Food Safety Authority and the Food and Drug Administration to ban the use of this chemical in many plastic products. Following such restrictions, several substitutes have been developed, with BPS and BPF representing the main replacements to BPA. While it has been demonstrated that BPA promotes oxidative damages in spermatozoa from different species, including human, the possible effects exerted by BPS and BPF on human sperm, have not yet been investigated.

Study design, size, duration: We explored the effect of 4 h *in vitro* exposure to scalar concentrations of BPS and BPF (from 10 to 400 μM), and to 400 μM BPA on sperm motility, viability, mitochondrial membrane potential (ΔΨm) and mitochondrial generation of reactive oxygen species (ROS). In a set of experiments, the effect of a combination of both BPF (400 μM) and BPS (400 μM) on Δ m and mitochondrial ROS generation was also assessed.

Participants/materials, setting, methods: Sperm ΔΨm was analyzed by flow cytometry with the fluorescent lipophilic cationic dye JC-1. Flow cytometric assessment of mitochondrial generation of ROS was carried out using the lipid soluble cation MitoSOX red (MSR). Sperm motility and viability were evaluated by Computer-Aided Semen Analysis (CASA) and eosin assay, respectively.

Main results and the role of chance: The exposure to scalar concentration of BPS did not significantly affect sperm motility and viability with respect to untreated controls. A lower, albeit not significant, sperm motility was registered in samples exposed to the highest concentrations of BPF (300 μM and 400 μM). As expected, 400 μM BPA produced a complete sperm immobilization along with a dramatically loss in sperm viability. No significant differences were observed in sperm ΔΨm and ROS generation after exposure to scalar concentration of BPS compared to untreated controls and the trend towards lower ΔΨm and higher mitochondrial ROS generation at the highest concentrations of BPF did not reach statistical significance. On the contrary, after 4 h exposure to 400 μM BPA a significant lower ΔΨm and higher mitochondrial ROS generation were observed. Finally, the exposure to a combination of BPF and BPS at high concentrations (400 μM) did not significantly affect sperm ΔΨm, or mitochondrial ROS generation, when compared to 400 μM BPA, used as positive control. Limitations, reasons for caution: The present study only evaluated BPS and BPF effects, but in daily-life people are exposed to several plasticizers containing different bisphenols at different concentrations. Therefore, adverse effects of synergic exposure to BPA analogues other than BPS and BPF, alone or in combination with BPA, cannot be ruled out.

Wider implications of the findings: The analogues BPS and BPF, alone or in combination, appeared to be safer alternatives to BPA on sperm biology as they exert a neutral effect on sperm motility, viability, and mitochondrial functions even at high concentrations. These results could be useful to identify more secure plasticizer components.

Trial registration number: Not applicable

P-049 The presence of double heads is associated with increased frequency of the other sperm abnormalities

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Study question: Is there an association between the presence of spermatozoa with double heads and the other sperm abnormalities in human semen?

Summary answer: Patients with double-headed spermatozoa had a significantly increased percentage of morphological abnormalities (head, midpiece and tail defects).

What is known already: The morphological evaluation of spermatozoa has a prognostic value for successful IVF procedure. It has been proven that certain morphological defects have a negative impact on fertilization, embryo quality, and pregnancy outcome in in-vitro fertilization cycles. Sperm abnormalities, such as double head, double tail and thin midpiece are rarely observed. However, their effect on the other sperm defects has not been well studied yet. The aim of this study was to examine the effect of the presence of double-headed spermatozoa on the frequency of occurrence of the other sperm defects.

Study design, size, duration: This retrospective study includes 2140 men aged between 18 and 73 years, with a mean of 36 years. It was conducted at Nadezhda Women's Health Hospital, Bulgaria between October 2015 and August 2020. A comparative analysis was performed between semen samples with and without double-headed spermatozoa and the other sperm abnormalities, as well as the percentage of morphologically normal forms.

Participants/materials, setting, methods: Morphological analysis was performed according to the Kruger's strict criteria. Totally 23 types of abnormalities were determined: head defects (small, large, amorphous, elongated, round, pear-shaped, double, acephalic, detached head, small and large acrosomal areas and spermatozoa without acrosome), midpiece defects (thick, bent, asymmetric, thin midpiece and cytoplasmic droplets), tail defects (stumped, coiled and double tail), acrosomal vacuoles, nuclear vacuoles and multiple defects. Statistics: Mann-Whitney U-test and T-test; $P \leq 0.05$.

Main results and the role of chance: Presence of double-headed spermatozoa was observed in 12.62% (270/2140) of the studied samples. In these

patients the frequency of occurrence of double-headed spermatozoa ranged between 1% and 29% with a mean of 0.41%±1.71%. Men with double-headed spermatozoa had significantly higher percentage of spermatozoa with small heads (24.51%±22.65%, P=0.04), round heads (11.69%±10.13%, P<0.01), nuclear vacuoles (10.64%±5.25%, P<0.01), sperm without acrosome (9.76%±8.61%, P=0.05), asymmetric midpiece (4.73%±3.96%, P<0.05), bent midpiece (8.9%±7.22%, P<0.01), thin midpiece (2.13%±4.44%, P<0.01), double tail (1.78%±0.8%, P<0.01), detached head (1.98%±1.42%, P<0.01), stumped tail (6.03%±5.19%, P=0.02), and cytoplasmic droplets (8.86%±5.02%, P<0.01) compared to the patients without double-headed spermatozoa. Moreover, the percentage of sperm with multiple defects in the double-headed group was significantly higher (35.53%±29.91%, P<0.01), while the percentage of normal forms was significantly lower (2.93%±3.64%, P<0.01) compared to the patients without double heads.

Limitations, reasons for caution: In this study unequal sample sized groups were compared. We also need to investigate whether the obtained results will be confirmed in patients with certain pathological states, such as oligozoospermia, teratozoospermia, and asthenozoospermia.

Wider implications of the findings: The present study revealed that the presence of double-headed spermatozoa in the ejaculate is related to an increased frequency of the other semen abnormalities. The double-headed spermatozoa could be used as an indicator for the total morphological quality of human spermatozoa

Trial registration number: not applicable

P-050 The effectiveness of the platelet-rich plasma treatment of men with severe oligoasthenoteratozoospermia

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Study question: To evaluate the effect of platelet-rich plasma (PRP) testicular injections on spermogram parameters of men with severe oligoasthenoteratozoospermia (OAT).

Summary answer: The PRP testicular injections have beneficial effects on spermatogenesis and enhance sperm concentration and motility in infertile men with OAT.

What is known already: The use of PRP therapy in assisted reproductive technologies is debatable. Despite the recent evidence of its positive effects in promoting endometrial and follicular growth, data from clinical studies are limited. There are only a few papers on the effectiveness of PRP therapy in the treatment of male infertility and sexual dysfunction. In more detail, the influence of PRP on spermatogenesis was carried out only on experimental animals. Although the mechanisms of its action have not yet been clarified, it is assumed that PRP, containing many biologically active molecules, realizes its effect through the tissue regeneration and cell proliferation.

Study design, size, duration: This prospective study included 68 men (34.6±5.2) years old with severe OAT (≤4 million/ml, motility ≤30%, normal sperm morphology ≤1%) receiving hormonal and antioxidant (AO) therapy during 6 months before in vitro fertilization cycles. 33 of them were injected once with autologous PRP (0.5 ml in each testicle). Spermogram and testosterone level were analyzed before the treatment and in 3, 4 and 6 months after it. Participants/materials, setting, methods: Sperm concentration, motility and morphology in ejaculate of 33 men of PRP group were compared with those in the group of 35 men without PRP within 6 months of starting the treatment. Total and free testosterone level were measured in blood serum. PRP was prepared by centrifuging the patient's own blood in the anticoagulant-containing tubes. The final concentration of platelets in the obtained sample was 950.000 – 1.250 000 cells in 1 ml.

Main results and the role of chance: 4 months after the PRP injection, sperm concentration and motility increased in 18 of 33 men of the PRP group compared with the baseline (before the treatment) – 4.2 (1.0; 6.9) vs 1.4 (0.1; 3.4) mln/ml (p<0.05) and 36.7 (30.6; 45.8) vs 17.7 (6.7; 28.2) % respectively (p<0.05).The maximum increase in sperm motility (but not in sperm concentration!) was observed in 24 men in 6 months – 49.6 (39.6; 56.4) % (p<0.05). Percent of morphologically normal spermatozoa in ejaculate slightly increased only in 12 men in that time period from 0-1 % to 1-2%. The total testosterone level was 2.4 times higher than the baseline (31.6±7.2 vs 13.2±4.3 nmol/l, p<0.05), the free testosterone level was 1.8 times higher (14.5±3.5 vs 7.9±3.0 pgl/ml, p<0.05).

Unlike the PRP group, in the group of men without PRP treatment, the sperm parameters did not changed compared with the baseline in 4 months after the starting hormonal and AO treatment. A significant increase of sperin concentration was observed only in 17 of 35 patients in 6 months. Sperm motility and percent of morphologically normal spermatozoa after the treatment did not differ from the baseline. Changes in the testosterone levels were similar to changes in PRP group.

Limitations, reasons for caution: Only young and middle-aged men were considered in the study. Large randomized controlled studies are required to confirm the PRP therapy efficacy and safety of f various fertility disorders. There are also no standardized protocols for PRP preparation.

Wider implications of the findings: PRP therapy may have great potential for the treatment of male infertility and improving spermatogenesis. Optimization of methods of PRP preparation and dosage of testicular injections can enhance reproductive outcomes in assisted reproductive technologies.

Trial registration number: not applicable

P-051 Differential resilience of sperm from different mammals to DNA decondensation

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Study question: Does sperm from different species with different protamine 1/protamine 2 ratios have different resilience to sperm decondensation?

Summary answer: Sperm cells from species whose DNA is condensed with both protamine 1 and protamine 2 require less time in deprotamination steps.

What is known already: Sperm cells present a highly particular DNA condensation that is acquired during sperm differentiation, where most part of histones are replaced by protamines. Protamines are key elements for DNA condensation and, while protamine 1 is more conserved among species, protamine 2 has evolved differentially, existing only a few species that retain the mature protein in their sperm DNA. Changes in protamine expression rates have been described to be associated to head sperm size and shape. In addition, reduced amounts of protamine 2 are related to male infertility in species in which this protein is present.

Study design, size, duration: Cryopreserved sperm samples were treated with lysis solutions to induce DNA decondensation and formation of sperm haloes. In these treatments, the effect of different incubation times with proteinase K added to the lysis solution upon DNA decondensation was tested by analyzing core diameter, halo diameter and the Halo/core ratio in at least 50 sperm per sample.

Participants/materials, setting, methods: Species included in the study were Human, Equine, Donkey, Porcine and Bovine. Sperm samples from five different individuals for each species were included in the study. DNA decondensation included three lysis steps: first, a SDS + DTT incubation for 30 minutes; second, a DTT + NaCl treatment for 30 minutes; and third, a DTT + NaCl + Proteinase K treatment with a variable time of 0, 30 or 180 minutes.

Main results and the role of chance: The halo/core diameter, used as a representation of the degree of DNA decondensation, for 0 minutes, 30 minutes and 180 minutes of proteinase K incubation were: 4.68±0.51, 4.32±0.51 and 4.77±0.64, respectively for human sperm; 4.15±0.41, 4.57±0.53 and 4.68±0.63, respectively for Equine sperm; 4.40±0.64, 4.00±0.37 and 4.17±0.19, respectively for donkey sperm; 1.77±0.2, 3.05±0.14 and 4.13±0.39, respectively for porcine sperm; and 2.40±0.40, 3.36±0.22 and 4.19±0.38, respectively for bovine sperm. Differences of halo/core ratio in different times were only observed in porcine and bovine sperm, where increasing degrees of DNA decondensation were found (p<0.05). Therefore, these results show that while longer incubations in lysis solutions with proteinase K lead to higher DNA decondensation in porcine and bovine, they do not induce higher decondensation in human, equine and donkey. This evidence, coupled to the fact that porcine and bovine sperm present

null or very low protamine 2 content, suggests that its presence might confer higher DNA decondensation susceptibility.
Limitations, reasons for caution: Only sperm cells with normal sperm haloes were analyzed in the present study. As multiple studies show, haloes exhibited by sperm cells with DNA damage display higher diameter, that is why they were strictly excluded in this study with the aim to elucidate the average DNA decondensation.
Wider implications of the findings: Sperm DNA might have different degrees of DNA condensation, which can be associated to a higher difficulty of DNA decondensation, thus having implications in the sensitivity tests that assess sperm DNA integrity.
Trial registration number: Not applicable.

P-052 Structural and functional changes in the prostate gland of men following orchitis

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Study question: The aim of the study was to establish echometric parameters, hemodynamic and cytohistological changes in the prostate gland in men of reproductive age after orchitis.
Summary answer: After orchitis, volume and mass of the prostate increased compared to the control group, blood flow was reduced, histologically and electron microscopy changes were observed.
What is known already: According to the literature, prostate diseases in men of reproductive age are an important issue in urology and andrology. The most common among them are infectious lesions of the genitourinary system, which constitute about 45%. These include, orchitis, as a part of the infectious process of the entire reproductive system. Half of the cases of orchitis are sexually transmitted infections or associated with infections that come from urogenital tract. In the majority of patients orchitis leads to infertility. But the state of the prostate, under these conditions, remains poorly understood.
Study design, size, duration: We used ultrasound diagnostics and colour ultrasound angiography of the prostate gland of 10 men aged 36-42 years, who suffered from orchitis. The data of 7 healthy men of the same age served as control. For histological and electron microscopy served tissues of prostate gland obtained from the 5 men of the same age group during minor invasive surgery. Statistical processing of the results was carried out using the program Statistica 10. Participants/materials, setting, methods: The length, width, height, volume and mass of the prostate were determined in the grey scale mode. The vascular pattern was determined by colour Doppler mapping (the course of the blood vessels, their diameter, the number in the symmetric sections of prostate). Qualitative hemodynamic indicators: peak systolic blood flow velocity (Vps) cm/s, diastolic blood flow velocity (Vd) cm/s, time average velocity (TAV) cm/s, pulsatility index (PI), volumetric flow rate (V) L/min. Histological methods and TEM.
Main results and the role of chance: After orchitis, the volume of prostate gland increased to (26.0 ± 1.4) cm3 vs (21.2±1.3) cm3 in control and its mass increased to (27.4±1.2) g vs (22.1±1.6) g in control group. The blood flow in the prostate was reduced: peak arterial blood flow velocity in the peripheral zone decreased up to (6.8±0.46) cm/s vs (18.8±3.0) cm/s in control and diastolic blood flow velocity decreased up to (2.75 ± 0.26) cm/s vs (5.7±0.1) cm/s in the control group. The final sections of the glands were cystically enlarged, the squamous epithelium was flattened, the nuclei were pyknotic and the cell borders were indistinguishable. Epithelial folds and shape were preserved, prostatic bodies and acidophilus secretion in the gaps were preserved. The relative volume of the glandular epithelium decreased up to 56.5% and the volume of the fibrous-muscular-elastic component around the lobules increased up to 43.5%. In the capillaries of the prostate, the nuclei of the endothelial cells were deformed, the cytoplasm was vacuolated, the crysts in the mitochondria were reduced, the basement membrane was expanded and uneven. In the nuclei of the prostatic epithelium the perinuclear condensation of chromatin observed, cytoplasm was vacuolated and accumulated drops of fat, the mitochondrial cristae were homogenized.
Limitations, reasons for caution: The results of the investigation approved by the Commission on Biomedical Ethics of the Precarpathian National University as appropriate and those do not violate moral and ethical norms in conducting research (Protocol 3 dated 16.10.2019).
Wider implications of the findings: The results of research indicated changes in the prostate, which require further investigation of hormonal balance in men under these conditions.
Trial registration number: *

P-053 Concentration of bisphenol S in human seminal plasma negatively correlates with decreasing sperm concentration in ejaculate

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Study question: Is there a relationship between concentration of bisphenol S in seminal fluid and spermiogram parameters?
Summary answer: Bisphenol S was detected in 81% of seminal plasma samples. Negative correlation was found between BPS concentration and total sperm count in normozoospermic men.
What is known already: Human spermatogenesis can be influenced by a range of chemicals present in our environment. Bisphenol S (BPS) is a very frequent compound commonly used as a softener in production of plastics, where it has replaced bisphenol A. It is an endocrine disruptor frequently associated with negative effects on reproduction. It has been observed that BPS can affect testicular development in rodent males. In addition, it has cytotoxic, reprotoxic and neurotoxic effects and induces the oxidative stress bringing negative effects on spermatogenesis. BPS has been detected in food, drinks or cosmetics. Its direct effect on spermatozoa or spermatogenesis is still unclear.
Study design, size, duration: A total of 38 patients (25 normozoospermic) aged 24 to 42 years, non-smokers, with BMI between 19,9-32,9 were included in this prospective study from 2018 to 2020. None of them had varicocele, urogenital infections or other urological problems. Their seminal plasma was separated by centrifugation. BPS was extracted using solvent extraction followed by preconcentration step. The samples were analysed on Agilent 6495 Triple Quadrupole. Two MS/MS transitions were used for quantitative LC-MS/MS analyses.
Participants/materials, setting, methods: All the men included in this study signed an informed consent and agreed with analyses of their samples. These analyses were approved by Ethical committee of University Hospital Brno. We evaluated the relationship between concentration of BPS in seminal plasma, sperm concentration, total sperm count, total motility, progressive motility, morphology and fragmentation of DNA in spermatozoa. Statistical evaluation was performed by one individual one-dimensional regression model (p-value lower than 0.05 were considered as statistically significant).
Main results and the role of chance: The examination revealed the presence of BPS in 31 samples of seminal plasma (81% of all the samples). In 6 samples, the concentration was under level of detection and in one sample under level of quantification. In 7 samples, a very high concentration was detected (>0.1 ng/ml). These values were then compared to spermiogram parameters and sperm DNA integrity. There were no significant differences between the concentration of BPS and morphology of spermatozoa, progressive motility and total motility. In case of the DNA integrity, the opposite trend was observed, lower proportions of spermatozoa with fragmented DNA were found in samples with higher concentrations of BPS. Evaluation of sperm concentration and BPS concentration showed relationship of increasing BPS concentration with significantly lower sperm concentration, the differences were most obvious when only the normozoospermic men were compared. Evaluation of BPS concentration and total sperm count revealed the same trend with statistically significant difference in the category of normozoospermic men. Due to the small number of samples, a negative effect of extreme values on the statistical evaluation cannot be excluded. Currently, more analyses focused on detection of BPS in seminal plasma are carried out in order to obtain sufficiently larger data set.

Limitations, reasons for caution: A limitation is the number of samples included and analysed in this study, which slightly reduced the power of statistical analysis.
Wider implications of the findings: These results document that BPS was present in 81% of analysed samples. Knowing the concentration of BPS in seminal fluid is important for understandig of impact of BPS on male fertility. Our future work will be focused on detection of other bisphenols in seminal plasma.
Trial registration number: MH CZ – DRO (FNBr, 65269705), AZV NV18-01-00544, Czech Ministry of Education, Youth and Sports (CZ.02.2.69/0.0/0.0/19_074/0012727)

P-054 Apoptosis related-microRNAs in Oligoasthenoteratozoospermia and Azoospermia men may reveal novel study of freezing damage

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Study question: Could choosing of non-apoptotic spermatozoa by biological biomarkers such as microRNAs promote post-thaw fertilization ability?
Summary answer: Biological alterations in correlation with apoptosis and oxidative stress markers such as microRNAs may preserve the function and fertility of spermatozoa during cryopreservation.
What is known already: Biological changes of cryopreserved spermatozoa such as microRNAs against cryo-injury were investigated. It was presented that several sperm parameters such as motility and abstinence period can impact the percentage of post-thaw sperm survival. Recent study, reported that microRNAs related to process of motility, sperm structure and apoptosis were associated with different expression after cryopreservation. More comprehensive study needed to fully mention the effect of microRNAs and their correlations with other biomarkers in cryopreservation.
Study design, size, duration: Our study was performed on 58 men who were 24-40 years old. Their ejaculated samples were classified as sever (concentrations less than 5 million sperm/mL) Oligoasthenoteratozoospermia (SOAT), mild (concentrations 5 million – 10 million sperm/ mL) Oligoasthenot eratozoospermia (MOAT), obstructive azoospermai (OA), Non obstructive azoospermai (NOA) (absence of spermatozoa in the semen) and normal group (concentrations more than 15 million sperm/ mL). Then each sample was grouped into fresh and cryopreserved one.
Participants/materials, setting, methods: Density Gradient centrifugation (DGC) was performed to obtain high quality sperm without round cells after freeze-thawing. Biopsy of testicular tissue was prepared after Testicular Sperm Extraction (TESE) surgery. Then biological biomarkers were examined before and after cryopreservation including microRNA-122 (miR-122), miR-383, miR-15b, miR-184, miR-34c and target genes such as P53, Caspase9 and CYCLIN D1, using Quantitative real-time polymerase chain reaction (RT-PCR). Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and malondialdehyde (MDA) using imaging multi-mode reader.
Main results and the role of chance: There was a significant reduction in sperm total motility and morphology in Cryopreserved-infertile groups (MOAT and SOAT) compared with the Fresh-infertile groups. Decreased level of GPx activity was associated with increased concentration of MDA during freeze-thawing procedure in oligoasthenoteratozoospermia. Also increasing levels of SOD, and DNA fragmentation were showed. Our data demonstrated that reduction of CYCLIN D1 in MOAT-Cryopreserved (P=0.0174) and NOA-Cryopreserved (P=0.0001) groups were considerable compared with their fresh ones. We observed high level of Capase9 and in cryopreserved groups (P=0.01).The expression of miR-34c was increased significantly in NOA-Cryo (P=0.0064), and OA-Cryo (P=0.0441) in comparison with their fresh groups. The expression of miR-184 (P=0.0275) was enhanced in NOA-Cryo as compared to NOA-Fresh. Quantitative RT-PCR demonstrated meaningful decrease level of miR-383 expression in SOAT-Cryopreserved as compared with SOAT-Fresh (P=0.0223). On the other hand, expression level of miR-383 was increased in NOA group significantly (P= 0.0437) and in OA group non-significantly during freezing. There was non-significant decrease of miR-122 and miR-15b in MOAT and SOAT-Cryopreserved groups in comparison to their Fresh groups. We observed reduced expression of miR-122 (P=0.0109) and miR-15b (P=0.0322) in OA group after freezing. Also, there was meaningful increased level of miR-15b (P=0.0234) in NOA-Cryo compared with Fresh.
Limitations, reasons for caution: Because of the ethical principle, we can not obtain testicular samples from normal groups. So, we analyzed NO and OA groups with each other.
Wider implications of the findings: Our study documented that total motility can be interfered by microRNAs. This phenomenon effects on the total motility of post-thaw spermatozoa. Also the increase level of MDA may disturb microRNAs regulation in the infertile cases. These non-coding RNAs may be known as fertility biomarker to development of freeze-thawing strategies.
Trial registration number: 60961

P-055 Methylation dynamics of the sperm epigenome after chemotherapy: a case study

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Study question: What is the evolution of the sperm epigenome after chemotherapy in a patient with testicular cancer (TC)?
Summary answer: These new data on epigenetic recovery profil after TC are useful tools for counseling and reassuring these patients.
What is known already: An important issue for young men affected TC is how TC and its treatment will affect, transiently or permanently, their future reproductive health. The consequences of cancer treatment on the sperm epigenome during the recovery periods are topical issues of ascendant significance as epigenetic modifications to the paternal genome may have deleterious effects on the offspring.
Study design, size, duration: Here we report the epigenomic profiling of frozen sperm from a TC patient before and after the treatment at different time points (6, 9, 12 and 24 months) by using RRBS analysis (Reduced representation bisulfite sequencing method).
Participants/materials, setting, methods: A testicular tumor (testicular germ cell tumor) was diagnosed in a 30 years old patient. A cryopreservation of spermatozoa was proposed before treatment.Semen samples were obtained 2 times before treatment and 4 times after treatment (6, 9, 12 and 24 months following the initiation of treatment).
Main results and the role of chance: Upon collection, sampling after chemotherapy ranged from 0,6 to 4,2 million per sperm straw between 6 and 24 months after the treatment, always increasing.

In order to capture the direct effect of the treatment on the methylation changes, the DMR detection has been operated between pre-chemotherapy samples (pair-wise) and the time point of 6 months. Among the 179 hqDMRs, 74 are differentially methylated between the PreCT and PostCT6m samples (16 hyper- and 68 hypo-methylated) associated with 49 DMGs (15 hyper- and 34 hypo-methylated).

We further sub-clustered the 74 hqDMRs between PreCT and PostCT6m into 6 patterns, 3 hyper- and 3 hypo-methylated. Briefly, patterns P1 and P4 include hqDMRs that quickly get back to their pre-treatment methylation status just after 9th months onwards. Patterns P2 and P5 include hqDMRs that slowly get back to their pre-treatment methylation status between 12 and 24 months after treatment. Patterns P3 and P6 include hqDMRs that remain hyper- or hypo-methylated even after 24 months.

We have intersected the genes (DMGs) associated with the detected hqDMRs with those known to be important or expressed during embryogenesis. We thus detected that 7 hyper-methylated and 6 hypomethylated DMGs were involved (or expressed) during embryonic / fetal development.
Limitations, reasons for caution: This study involves a single patient. As the patient made no major changes in his personal way of life, we hypothesized that sperm parameter variations may be attributable to the BEP treatment.
Wider implications of the findings: The altered methylated status of those DMGs important for early development might modify their expression pattern and thus affect their function during key stages of embryogenesis leading to

potential developmental disorders. It is important to notice that among the 110 DMGs none of them correspond to known imprinted genes.
Trial registration number: not applicable

P-056 To graft or not to graft? Intratesticular grafting of testicular tissue from Klinefelter boys to the mouse testis as possible novel in vivo model

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Study question: Can intratesticular transplanted testis tissue from Klinefelter boys to the mouse testis be used to study the mechanisms behind testicular fibrosis?
Summary answer: Grafting of testicular tissue from Klinefelter boys to the mouse testis is not a valuable new *in vivo* model to study Klinefelter-related testicular fibrosis.
What is known already: Klinefelter syndrome (KS; 47, XXY) affects 1-2 in 1000 males. Most KS men suffer from azoospermia due to a loss of spermatogonial stem cells. Additionally, testicular fibrosis is detected from puberty onwards. However, mechanisms responsible for fibrosis and germ cell loss remain unknown. An optimal *in vivo* model to study the KS testicular fibrotic process is not available.

This study aimed to evaluate a possible *in vivo* model to study KS-related testicular fibrosis. In addition, the effect of the mast cell blocker ketotifen, which showed positive effects on fertility in infertile non-KS patients, was evaluated in this graft model.
Study design, size, duration: First, the survival time of the KS graft was established, since it was the first time KS tissue was transplanted to the mouse testis. Testes were collected after two, four, six and eight weeks after which histological and immunohistochemical evaluations were performed. Next, the effect of daily ketotifen injections on the fibrotic appearance of intratesticular grafted testicular tissue from KS and controls was evaluated.
Participants/materials, setting, methods: Testicular biopsy samples from pre- and peripubertal KS (n=22) and age-matched control samples (n=22) were transplanted to the testes of six weeks old Swiss Nu/Nu mice (n=22). Prior to grafting, testicular tissue pieces were cultured in vascular endothelial growth factor (VEGF) for five days. Next, tissues were transplanted to the mouse testes. Testicular transplants were analysed by immunohistochemistry. In the second experiment, mice were given daily subcutaneous injections of ketotifen or saline.
Main results and the role of chance: Four weeks after transplantation, all KS grafts could still be retrieved. At a later timepoint, degeneration of the tissue could be detected. In the grafts, recovered four weeks after transplantation, about 30% of the tubules in peripubertal grafts showed a good integrity, while in the prepubertal tissue, 83% of the tubules were intact. A fibrotic score was assigned to each graft. No significant changes in fibrotic score was observed between testicular biopsies before or after transplantation. However, an increased ($p<0.01$) fibrotic score was observed after in-vitro treatment with VEGF both in control and KS tissue. Based on recovery and tubule integrity grafts were recovered after four weeks in the second experiment. Treatment with ketotifen did not result in significant histological differences compared to non-treated grafts (KS and control tissue).

The survival potential of grafts from KS testicular biopsies of pre- and peripubertal boys was patient- and age-dependent. After four weeks, most KS tissue starts to degenerate. In prepubertal tissue, seminiferous tubules were mostly intact, while tissue from adolescent boys was impaired. Interestingly, no loss of germ cells was observed after transplantation of the testicular tissue.
Limitations, reasons for caution: The availability of tissue from young KS patients is very scarce, leading to a low number of included patients (n=8). Testicular tissue pieces from the same patient were included to evaluate the differences before and after transplantation. However, histological variability between testicular tissue biopsy pieces is well-known in KS patients.
Wider implications of the findings: Since testicular tissue from KS boys, transplanted to the mouse testes, already starts to degenerate after four weeks and the integrity is not optimal, we conclude that this is not a valuable model for future studies. *In vitro* models to study the KS-testicular fibrosis should be investigated.
Trial registration number: NA

P-057 Preliminary evaluation of laser confocal Raman spectroscopy as a noninvasive method for detecting sperm chromosome aneuploidy

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Study question: To evaluate the efficiency and accuracy of Raman microspectra in detecting sperm chromosome balance state by DNA content difference.
Summary answer: Raman spectroscopy can identify the difference of X and Y sperm DNA content, but the accuracy still needed to be improved for clinical application.
What is known already: Aneuploid sperm fertilization affects embryo quality and leads to the waste of oocytes in Assisted Reproductive Technology (ART). Raman spectroscopy can identify substances and observe molecular changes through specific spectral patterns with high specificity and has become a new hot spot in ART. Previous research has used this technology to detect embryo culture medium to evaluate the aneuploidy of embryos. The DNA content of X and Y in sperm was different, which may serve as a marker for sperm aneuploidy detection by Raman spectroscopy.
Study design, size, duration: The significant difference in the morphology of the sex chromosomes of X and Y spermatozoa leads to a substantial difference in the DNA content. We perform Raman spectroscopy to identify the spectral differences of the sperms, especially the differences in sperm DNA content. We further verified the accuracy with fluorescence in situ hybridization (FISH).
Participants/materials, setting, methods: Spermatozoa were provided by healthy donors with normal aneuploidy, and analysis parameters met the current World Health Organization (WHO, 2010) standards. Sperm heads were detected by laser confocal Raman spectroscopy and obtained the corresponding spectra. The sperm chromosome information was classified by Standard principal component analysis (PCA) and identified by fluorescence in situ hybridization (FISH). Student's t-test and Receiver operating characteristic (ROC) curve analysis was performed for further analysis.
Main results and the role of chance: Standard principal component analysis (PCA) after unqualified quality control divided spermatozoa into two groups according to the calculation and calibration results, 22 cases in group A and 31 cases in group B. Then, we conducted frequency distribution histogram statistics on the above data, and the results showed that there were differences in frequency distribution at I785 = 23,750 and Area714 -1162 = 3,250,000. The FISH analysis identified sex chromosomes of 59 spermatozoa, which was not exactly one-to-one correspondence with the results of PCA analysis. Then we further analyzed the sperm of 59 cases by statistical analysis. The results showed that there were significant differences between X sperm (n = 39) and Y sperm (n = 20) at 714-1162 cm-1 and 785 ($P<0.05$). ROC curve analysis was used to evaluate the sensitivity of correlation between sperm DNA content and Raman spectra. The results showed that the corresponding thresholds of I785 = 24,986.5 and Area714-1162 cm^{-1} = 3,748,990 were the best for distinguishing the two kinds of sperm. When the sperm's peak value of 785 or 714-1162cm^{-1} exceeds the above thresholds, X-sperm's possibility greatly increased. The AUC of the ROC curve in both cases was 0.662 and 0.696, respectively.
Limitations, reasons for caution: Current Raman spectroscopy requires spermatozoa elution and fixation, which damage the sperms. Furthermore, current Raman spectral data are not obtained from the whole sperm head, limiting the accuracy of this technique.
Wider implications of the findings: Our results indicated that Raman spectroscopy had potential application value for sperm aneuploidy detection and could be used as a noninvasive selector for normal haploid sperms in the ART.
Trial registration number: LL-SC-2018-038

P-058 The effect of sperm DNA fragmentation on intracytoplasmic sperm injection (ICSI) outcome

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Study question: Does the sperm DNA fragmentation (SDF) level impact the clinical outcome of couples undergoing intracytoplasmic sperm injection (ICSI)?
Summary answer: No significant effect was observed for SDF on the reproductive outcome of couples undergoing ICSI.
What is known already: Sperm DNA Fragmentation (SDF) has emerged as an important biomarker in the assessment of male fertility potential. It is currently being used as one of the advanced sperm function tests along with other conventional methods in male fertility evaluation. The impact of SDF on the reproductive outcomes of ICSI remains to be controversial. Evidence extracted from three meta-analyses have indicated that higher SDF is not associated with a negative impact on ICSI outcomes. On the contrary, another meta-analysis revealed that SDF can have a significant impact on the pregnancy rate of ICSI with an OR of 1.31.
Study design, size, duration: This is a retrospective cohort study carried out in the assisted conception unit of a tertiary medical center. The study duration was over a 5-year period from August 1st, 2014 to August 1st, 2019. The charts of 1922 patients who underwent ICSI were screened for inclusion in the study. Inclusion criteria were patients who underwent ICSI using ejaculate spermatozoa and had a recorded SDF test done within a week before ICSI (n=390).
Participants/materials, setting, methods: Sperm chromatin dispersion was used to evaluate SDF utilizing the Halosperm G2 test kit (Halotech, Madrid, Spain). All patients performed the ICSI trial using ejaculated spermatozoa. Patients were divided according to the SDF level into 3 groups; SDF <20% (n=148), SDF 20-30% (n=133), and SDF >30% (n=109). Female partner fertility status was recorded and couples were grouped into 2 groups based on age and AMH levels; (1) favorable female and (2) unfavorable female status.
Main results and the role of chance: Overall, clinical pregnancy occurred in 45% of cases, live birth rate was 33.60%, and 1.30% of patients had miscarriage. A significant negative correlation between SDF and sperm count (r-0.232), motility (r-0.469), progressive motility (r-0.312) and normal morphology (r-0.297) was detected (p<0.001 for all). Fertilization rate, clinical pregnancy and live birth rate were greater in patients with lower SDF than those with higher SDF in both favorable and unfavorable groups, however the difference was not statistically significant (Table 1).
Limitations, reasons for caution: The main limitation of our study was the retrospective nature of the study where some data may be missing or incomplete. The data was also retrieved from one ART center, therefore our data lacked diversity within methodologies for IVF and SDF testing.
Wider implications of the findings: SDF was found to be significantly correlated with conventional semen parameters highlighting its significance as a robust diagnostic test during male fertility evaluation. In this study, while patients with higher SDF values had worse reproductive outcomes with ICSI, the results did not reach statistical significance.
Trial registration number: NA

P-059 Association between seminal levels of Fe and semen quality

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Study question: The detection of metals in semen offers a new field in the study of male infertility.
Summary answer: Normozoospermia is associated with higher amounts of Fe. In males with pathological spermiogram, the percentage of men with Fe in semen was lower than expected.
What is known already: Increased levels of Fe in human semen appear to have a significant correlation with male fertility, suggesting that Fe in human seminal plasma has an important factor in male reproductive function. Fe acts as an antioxidant being a co-factor of catalasa, which protects sperm. On the other hand, elevated Fe levels are associated with sperm damage and continues to increase the lipid peroxidation that will affect the plasma membrane and the sperm motility. Most authors associate Fe with sperm motility and higher estimated fertility potential, based on standard semen parameters in fertile men, which are associated with lower levels of Fe.
Study design, size, duration: A prospective study was carried out in 102 men in a Reproduction unit in Tenerife, from February to April 2018 as a part on an epidemiologic study of environmental contaminants and male reproduction. The participant were categorized into two groups, according to the results of semen analysis following the World Health Organization guidelines: the pathological and the normal semen group that constituted the control group. The metal was determined in semen samples.
Participants/materials, setting, methods: Semen quality and levels of Fe were measured in seminal plasma on a total of 102 men attended successively, for the initial infertility evaluation, The collected samples were used for both semen analysis following the World Health Organization (WHO) guidelines and metal detection and carried out using a Makler® counting chamber (Irvine Scientific, CA) and for metals, were determined by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) in semen samples.
Main results and the role of chance: The percentage of males with the presence of Fe was 97.1% and the average level were 0.6283 mg/Kg. When analyzing the relationship between the spermiogram parameters with the levels of Fe in the semen, significant differences were found. All men with a normal sperm analysis presented Fe in semen, but among men diagnosed with altered spermiogram, the percentage of men with Fe in semen (92.7%) was lower than expected (97%) (2 128 1 =4.59; *p* =0.032). As for the concentration of Fe in spermiogram in the first quartile (25% lower), measuring 0.33 mg/Kg, more pathological samples were found than expected (χ2 133 2 =6.921; *p*=0.031) having a higher probability of being more pathological (52% vs 31.4%). On the other hand, men with pathological sperm concentration, have higher levels of Fe, in the fourth quartile (0.61 mg/kg), with more frequency than expected (90.6% vs 97%) (χ2 136 1 =6.48; *p*=0.011). The association between BMI and the presence of Fe was statistically significant. In obese males (BMI ≥30.0 kg/m2),

	Favorable group			Unfavorable group		
SDF	**<20**	**20-30**	**>30**	**<20**	**20-30**	**>30**
Fertilization rate	75%	77.8%	73.03%	71.4%	75%	80%
Clinical pregnancy	52.2%	51.2%	48.5%	34%	39.2%	31.7%
Miscarriage	1%	1.2%	2.9%	0%	0%	2.4%
Live birth rate	50%	42.9%	41.9%	26.1%	33.3%	16.7%

p value >0.05 in all.

the percentage of men with Fe in semen (80%) was lower than expected (97%) ($\chi 2\ 42\ 2 = 11.302$; $p = 0.001$).
Limitations, reasons for caution: The limitation of this study was the volume of semen that could be obtained for metal detection, only 0.8 mL. because the collected samples were used for both semen analysis and metal detection.
Wider implications of the findings: The determination of metals in semen opens a new field in the study of male infertility and many cases of unknown infertility could be due to metal presence or absence in semen, with the option of performing treatments.
Trial registration number: not applicable

P-060 Dose- dependent mitigation of lead acetate toxicity in males on embryo development in female mice

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Study question: Does quercetin (75 or 100 mg/kg BW/day) co-administration with lead acetate to male mice affects embryonic development in female mice?
Summary answer: The low-dose quercetin (75 mg/kg BW/day) ameliorated the adverse effects of lead acetate on mouse embryogenesis.
What is known already: Lead causes male infertility by impacting on endocrine system and spermatogenesis, and may exert undesirable effects on the offspring. The currently approved treatment for lead poisoning is the use of chelating agents, which form an insoluble complex with lead and shield it from biological targets; thus, reducing its toxicity. One of the main mechanisms of lead-induced toxicity is oxidative stress, and it has been reported that natural antioxidants can reduce the heavy metals toxicity. The aim of the present study was to examine the protective effects of quercetin on the toxicity induced by lead acetate on the embryogenesis in mice.
Study design, size, duration: Sexually mature (eight-week-old) NMRI male mice (n= 24) were randomly divided into four groups (n= 6 per group) receiving (i) distilled water (control group); (ii) lead acetate (150 mg/kg BW/day) dissolved in deionized water (LA); (iii) lead acetate (150 mg/kg BW/day) + quercetin (75 mg/kg BW/day) (LQ75); (IV) lead acetate (150 mg/kg BW/day) + quercetin (100 mg/kg BW/day) (LQ100). Treatments were applied daily as oral gavages for one cycle of the seminiferous epithelium (35 days).
Participants/materials, setting, methods: At the end of treatment administration, the males were joined with super-ovulated females, and the retrieved zygotes were cultured for evaluation of the embryo development (at 2-cell, 4-cell, 8-cell, and blastocyst stages), and blastocyst cell number using differential staining (propidium iodide and bisbenzimide). After incubation of capacitated sperm with oocytes, an ultraviolet light microscope was used following 3 min incubation with 25 μg⁄mL bisbenzamide solution for fertilization assessment.
Main results and the role of chance: Lead acetate (LA) treatment of male mice decreased the 2-cell stage compared with the control group ($P > 0.05$). There was no difference between control and LQ75, and between LA and LQ100. The other stages of embryonic development were not significantly affected by the treatment. Overall, early embryonic development in the control and LQ75 mice were better than LQ100 and LA mice.

The number of cells in the trophectoderm and inner-cell mass were not affected by treatments. However, the total blastocyst cell number in the control was higher than in the other groups; there was no significant difference between LQ100, LQ75 and LA groups. Fertilization rate was not affected by the treatments ($P < 0.05$).

Quercetin acts as a potent antioxidant at low doses, but at high doses exerts a pro-oxidant action. According to previous reports, higher concentrations of quercetin increased apoptosis and necrosis while decreasing the activities of the antioxidant enzymes. Also, it has been suggested that quercetin might disrupt the endocrine system and interfere with Sertoli cell function and sperm motility.
Limitations, reasons for caution: A limitation of this study is narrow dose selection; more studies are needed to determine the effective dose of quercetin in ameliorating the lead toxicity. There are also side effects of lead-quercetin chelates such as metal redistribution, essential metal loss, accumulation and persistency in intracellular sites, and peroxidation.
Wider implications of the findings: Lead administration adversely impacted on the embryogenesis; on the other hand, paternal quercetin co-administration somewhat ameliorated the adverse effects of lead on mice embryogenesis.
Trial registration number: Not applicable

P-061 Protective effect of melatonin against bleomycin, etoposide, and cisplatin (BEP) chemotherapy-induced testicular toxicity in Wistar rats: A biochemical, immunohistochemical and apoptotic genes based evidence

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Study question: Does exogenous melatonin (MLT) attenuate BEP-induced damage in testicular cells and spermatogenesis in a dose-dependent manner?
Summary answer: Melatonin protected the testes against BEP-induced testis damage through ameliorating nitro-oxidative stress, apoptosis, and inflammation. However, there was no significant difference between melatonin-treated groups.
What is known already: Recently, the prevalence of testicular cancer (TC), accounting for the most common cancer among young people of reproductive age (15–40 years), has risen internationally. BEP chemotherapy has increased the 5-year survival rate of TC patients at all stages of testicular germ cell tumors to 90-95%. However, BEP creates a high incidence of male infertility and even long-term genotoxic effects, which emerges as a critical health issue. Melatonin is a well-known potent antioxidant with widespread clinical applications that recently has been giving increasing attention to its role in male sub/infertility.
Study design, size, duration: 60 Adult male Wistar rats were randomly assigned to six groups (n=10/group). Group 1, 3, and 4 were injected with vehicle, 10 and 20 mg/kg of melatonin, respectively. Other groups received one cycle of bleomycin, etoposide, and cisplatin for a total of 3 weeks with or without melatonin. Melatonin administration started daily one week before BEP initiation continued on days 2, 9, and 16; and one week after the completion of the BEP cycle.
Participants/materials, setting, methods: Bodyweight, testes weight, Sperm parameters (count, motility, viability, and morphology), testosterone hormone level, testicular histopathology, stereological parameters, testicular level of malondialdehyde (MDA), nitric oxide (NO), and total antioxidant capacity (TAC), the expression of Bcl-2, Bax, Caspase-3, p53, and TNF- (Real-time PCR and immunohistochemistry) were evaluated at the end of the study (day 35).
Main results and the role of chance: Our findings showed that melatonin restores the BEP-induced reduction in the body and testes weight (P<.05). the evaluation of quantitative analysis of the testes stereological procedures, QRT-PCR examination and immunohistochemical (IHC) staining revealed that melatonin reverses the BEP-induced impaired spermatogenesis (P<.05). Furthermore, melatonin rectifies BEP-induced disturbance on sperm count, motility, viability, and morphology. The testosterone level in the BEP-treated group was decreased significantly by comparison with the control group (P<.01). By contrast, co-administration of 10 and 20 mg/kg of melatonin could enhance the serum testosterone level significantly (P<.05). Moreover, melatonin enhanced the antioxidant status of the testis by elevating TAC and ameliorating MDA and NO levels. More notably, QRT-PCR examination indicated that melatonin therapy suppressed BEP-induced apoptosis by modulating apoptosis-associated genes such as Bcl-2, Bax, Caspase-3, p53 in the testis (P<.01). Besides, Co-administration of 10 and 20 mg/kg of melatonin with BEP regimen decreased significantly the population of p53 (54.21 ±6.18 % and 51.83±8.45, respectively) and TNF- positive cells (42.91±9.92 % and 33.57±2.97, respectively) by comparison to the BEP group.

Also, melatonin with low and high doses could enhance the expression of Bcl-2 protein in spermatogenic cells line (59.19±10.18 %, 63.08±5.23, respectively) compared to the BEP-treated group.
Limitations, reasons for caution: Owing to limited laboratory facilities we were not able to perform further studies to verify the mechanism of melatonin in the specific targets by using transfection technique and transgenic.
Wider implications of the findings: These findings can draw attention to the clinical application of melatonin and also suggest that melatonin may be an attractive agent for attenuating chemotherapy-associated male sub/infertility. This indolamine also may shorten the fertility recovery period in patients undergoing chemotherapy with the BEP regimen.
Trial registration number: N/A

P-062 Annexin-V MACS method could be a good choice for sperm selection with high PLCZ1 expression and high blastocyst rate in high DFI male factor

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Study question: What effect does Annexin-V MACS method have on sperm selection by pLCZ1 expression and blastocyst rate in male DNA fragmentation?
Summary answer: AnnexinV-MACS method could be a good choice for sperm selection with high pLCZ1 expression and high blastocyst rate in high DNA fragmentation male factor.
What is known already: Sperm selection based on morphology and motility in ART techniques, is not enough for choosing the best sperm especially in male factor patients. In Annexin-V magnetic activated cell sorting (MACS) technique, apoptotic sperm are separated from non-apoptotic one by negative selection. So, this method can improved quality of compaction rate in embryo. PLC is oocyte activating factors that it starts oscillations of calcium ca2+ in oocyte and it has a significant effect on fertilization and implantation.
Study design, size, duration: Semen samples from 30 male factor infertile couples with high DFI (DFI>30%) were selected and divided into two group (control and experimental) in each patient.
Participants/materials, setting, methods: Control was washed with DGC and experimental one was selected by MACS-DGC. Retrieved eggs in each patient, were divided in 2. Both group were injected by DGC and MACS respectively. Semen parameters and DFI (SCD test) were analyzed before and after processing. After ICSI, rate of fertilization, embryo development and blastocyst formation were evaluated. Real time PCR evaluate expression of PLC . Comparison between results of two groups was assessed by SPSS analysis.
Main results and the role of chance: Results showed that, sperm motility and morphology after MACS method (45%, 1.7%) was significantly higher than DGC method (40%, 1.1%) and before washing (35%, 0.9%). Percent of DFI in MACS group (36%) was significantly decreased compared to DGC (45%) and primitive group (55%). The number of oocytes were injected in DGC group was 93 and in MACS group was 111. Fertilization rate in both groups was almost the same (72.07% in MACS vs 73.11 in DGC). Rate of day 3 embryo with good grade in MACS group (72. 5%) was significantly higher than DGC (51.47%) (P<0.05). The blastocyst rate in MACS-DGC group (69.69%) was significantly increased compared to DGC group (48%). PLCζ gene expression in MACS-DGC was significantly higher than DGC group (p-value=0.046).
Limitations, reasons for caution: This experiment was performed invivo.
Wider implications of the findings: Sperm selection by MACS-DGC method can improve sperm motility, morphology and reduce sperm DFI. No significant difference was observed in fertilization rate, but percent of high-quality embryo on days 3 and 5 was significantly higher by this method,also it can be suggested as a good choice for patients with highDF.
Trial registration number: not applicable

P-063 The influence of lifestyle factors on sperm quality by motile sperm organelle morphology examination (MSOME)

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Study question: This study aimed to investigate the influence of lifestyle factors on sperm quality according to Motile Sperm Organelle Morphology Examination (MSOME) criteria.
Summary answer: The introduction of MSOME permits the examination of subcellular defect like nuclear vacuoles at hight magnification (6000x) in real time on vital sperm.
What is known already: It is increased accepted that lifestyle factors have an impact on sperm quality. Recent evidence shows that the selection of spermatozoa based on the analysis of morphology under high magnification may have a positive impact on embryo development in cases with severe male factor infertility and/or previous implantation failures. Therefore, MSOME has been considered as representing an improvement in the evaluation of semen quality. Although numerous studies have shown the influences of nutrition, lifestyle, age on semen quality, only very few study, have considered the influence of these factors on the vacuolization rate in semen analysis (MSOME criteria)
Study design, size, duration: The objective of this prospective study was to compare the semen parameters of 87 male patients undergoing evaluation or treatment of infertility at Unit of Pathophysiology of Reproduction ad PMA-Santa Maria Goretti Hospital -Latina according to MSOME and WHO (World Health Organization) criteria between January and September 2019.Written informed consent was obtained from all partecipant of this study.
Participants/materials, setting, methods: The subjects were divided into three groups according to age: Group I ≤ 35 years, group II ,36-40 years; and Group III ≥ to 41 years .All patients filled a questionnaire answering questions regarding age, BMI, caffeine and alcohol consumptions, smoking and nutrition behavior. Were excluded from the study patients with chromosomal alteration. For multifactorial lifestyle influence patients were evaluated with a point base system with a cut-off >2 and cut off<3 for unhealthy style.
Main results and the role of chance: There was no difference between the groups with regard all semen parameters such as volume, concentration, number of leukocytes, morphology and vitality (%).The percentage of spermatozoa with LNV (Large Nuclear vacuoles) was significantly higher in the older group than in the younger (I and II) (39,14±13,74 vs 31,8±12 and 31,7 ±13,4 rispectively (p<0,05)which does not correspond to a worsening of semen morphology. Regression analysis demonstrated a correlation between the percentage of spermatozoa with LNV and male age (r = -0,1) (p<0,001). There was no correlation between lifestyle parameters ad enviroments factors . Comparing the semen parameters of healthy and unhealthy population we found no difference except a significantly higher number of spermatozoa with vacuoles in the unhealthy population (p <0.001)
Limitations, reasons for caution: Although the sample examined in this study is limited in size and other studies are needed to confirm this evidence, the data available to us support the routine use of MSOME for ICSI and as a criterion for semen analysis with potential clinical repercussions.
Wider implications of the findings: To date, there are few works in the literature that analyze the relationship between the morphology assessed with the MSOME and the age of the patients and the results are conflicting. To our knowledge many works agree with our results.
Trial registration number: not applicable

P-064 Application of an artificial intelligence model for morphologic prediction of fertilization-competent human spermatozoa

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Study question: What is the role of artificial intelligence in selecting fertilization-competent human spermatozoa according to their morphological characteristics? Summary answer: The established AI model in this study can be potentially used to select semen samples with superior fertilization potential in clinical settings.
What is known already: Defective spermatozoa-zona pellucida (ZP) interaction causes subfertility and is a major cause of low IVF fertilization rates. While ICSI benefits patients with defective spermatozoa-ZP binding, a standard method

to identify such patients prior to conventional IVF is lacking. The application of artificial intelligence to sperm morphology analysis has become a topic of growing interest owing to the fact that the conventional assessment is highly subjective and time-consuming. Deep-learning, a core element of artificial intelligence (AI), incorporates the convolutional neural networks (CNN) to process all the data composing a digital image through successive layers to identify the underlying pattern.
Study design, size, duration: The fertilization-competent spermatozoa were isolated according to their binding ability to the ZP. The ZP-bound and -unbound spermatozoa were collected for functional assays and to establish an AI model for morphologic prediction of sperm fertilization potential. Human spermatozoa (n=289) were isolated from normozoospermic samples. Human oocytes (n=562) were collected from an assisted reproduction program in Hong Kong. Sample collection has been ongoing and will continue until the end of this study in November 2021.
Participants/materials, setting, methods: Sperm-ZP binding assay was employed to collect ZP-bound and -unbound spermatozoa. The fertilization potential and genetic quality of the collected spermatozoa were evaluated by our established protocols. Diff-Quik- stained images of ZP-bound and -unbound spermatozoa were collected respectively for the establishment of an AI model. A novel algorithm for sperm image transformation and segmentation was developed to pre-process the images. CNN architecture was then applied on these pre-processed images for feature extraction and model training.
Main results and the role of chance: Our result showed that the sperm-ZP binding assay had no detrimental effect on sperm viability when compared with the raw samples and unbound-sperm subpopulations. ZP-bound spermatozoa were found with statistically higher acrosome reaction rates, improved DNA integrity, better morphology, lower protamine deficiency and higher methylation level when compared with the unbound spermatozoa. A deep-learning model was trained and validated by analyzing a total of 1,334 and 885 of ZP-bound/ unbound spermatozoa to evaluate the predictive power of sperm morphology for ZP binding ability. Our newly trained AI-based model showed initial success in classifying the ZP-bound/ unbound spermatozoa according to their morphological characteristics with high accuracy of 85% and low computational complexity.
Limitations, reasons for caution: This sperm selection method requires micromanipulation and relatively long processing time to recover ZP-bound spermatozoa. In addition to limited availability, the use of human materials may result in interassay variations affecting the reproducibility of this method among laboratories.
Wider implications of the findings: In light of current findings, AI-based sperm selection method may provide high predictive values of sperm fertilization potential for clinical purposes. This method is particularly applicable to patients who had poor fertilization outcomes after conventional IVF treatments or those with high degree of defective sperm-ZP binding ability.
Trial registration number: not applicable

P-065 Advanced paternal age does affect egg donation program outcomes?

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Study question: Does increasing paternal age has a negative impact in fertilization (FR), blastulation (BR), clinical pregnancy (CPR) and miscarriage (MR) rates in an egg donation program?
Summary answer: The increase paternal age in an egg donation program has not a negative impact in fertilization rate, blastulation rate, clinical pregnancy rates and miscarriage rates.
What is known already: It is well documented that semen quality is affected with increasing paternal age but there is no evidence-based definition of what is advanced paternal age. There is controversial information about if the increasing paternal age affects in vitro fertilization results, and when this negative impact could begin.
Study design, size, duration: This was a single center retrospective cohort study, involving 485 first single embryo transfer of an egg donation program, from January 2017 to December 2019.
Participants/materials, setting, methods: All first embryo transfer of egg donation cycles performed at CEGyR, Buenos Aires, Argentina were included. Elevated sperm DNA fragmentation (TUNEL >20), sperm bank, and testicular biopsy cycles were excluded. Patients were divided according to male partner age: (1) <41, (2) 41-44, (3) 45-50 and (4) >50 years old. Group (1) was considered the control group. Statistical analyses were performed for FR and BR with ANOVA and CPR and MR with chi-squared tests.
Main results and the role of chance: The number of patients in group (1) was 200, in (2) 130, in (3) 117 and in (4) 38. Male average age was 36,8 in group (1), 42,2 in (2) 47,1 in (3) and 54,2 in group (4). The FR in group (1) was 72,60%, in group (2) was 73%, in (3) was 75% and 73% in (4). ANOVA results for FR: F=0,65 (p: 0,58). The BR, defined as the relation between the total number of blastocysts over the number of fertilized oocytes in a cycle, was in group (1) 46,35%, in group (2) was 45%, in group (3) 46%, and in group (4) 42%. ANOVA results for BR F=0,36 (p:0,78). The CPR in group (1) was 42,19%. Comparing with the other groups: group (2) was 37,09% (chi-square statistic=0,64 p:0,43); group (3) 34,58% (2,32 p:0,13); and group (4) was 32,43% (1,48 p:0,22). The MR in group (1) was 12,49%. Comparing with the other groups: group (2) was 18,55% (chi-square statistic=2,31 p:0,12); group (3) 14,94% (1,01 p:0,32); and group (4) was 15,85% (0,91 p:0,33). For all results analyzed there were not a statistically difference between groups.
Limitations, reasons for caution: The main limitation of this study was its retrospective design based on data from a single center which may be subject of bias.
Wider implications of the findings: Further large prospective studies are required to make meaningful comparisons. Our findings give no support for a general recommendation.
Trial registration number: not applicable

P-066 Does microfluidic sperm sorting (MSS) affect embryo euploidy rates in couples with high sperm DNA fragmentation (SDF)?

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Study question: Does MSS (microfluid chip-sorted spermatozoa selection) provide improvement on embryo quality and euploidy rates in couples with high SDF (sperm DNA fragmentation) and previous failed in vitro fertilization/ intracystoplasmic sperm injection (IVF/ICSI) cycles?
Summary answer: Use of MSS technique provides higher number of top quality blastocysts compared to density gradient centrifugation (DGC), however euploidy and live birth rates weren't improved.
What is known already: Previously it has been reported that sperm DNA damage leads to poor embryo development and there is a significant association between SDF and high embryo aneuploidy rates. Recently this fact raised attention to sperm selection techniques such as MSS to enhance embryo quality, miscarriage rates and embryonic euploidy rates.
Study design, size, duration: This was a retrospective electronic medical record (EMR) analysis of a tertiary assisted reproduction center between 2016 and 2020. All EMR were reviewed to select eligible cases as; couples undergoing a new IVF/ICSI cycle with PGT-A (preimplantation genetic testing for aneuploidies). In total, data from 243 patients were obtained for the analysis that accounts for 688 embryos.
Participants/materials, setting, methods: Patients had at least 2 previous failed IVF cycles and males had at least 20% SDF. In their new cycles, MSS was offered, preceding ICSI and PGT-A. Couples who accepted the technique were assigned to MSS group (92 cycles with 310 embryos) and the rest were managed with DGC and assigned as controls (151 cycles with 378 embryos). Azoospermia cases and women with age>43, uterine abnormalities, trombophilia were excluded. Biopsies were performed at blastocyst stage.
Main results and the role of chance: Two groups were comparable in terms of demographic data including women and men age, SDF, sperm parameters and cycle characteristics. There was no difference between groups in terms of fertilization rates (MSS 85% vs DGC 79% p=0.9), euploidy rates (MSS 53.2% vs DGC 50.7% p=0.3), mean no of euploid embryo per patient (MSS 1.09 vs DGC 0.95 p=0.3), positive pregnancy test (MSS 50% vs DGC 38.4% p=0.06), clinical miscarriage (MSS 7.6% vs DGC 6.6% p=0.7) and live birth rates (LBR)(MSS 42.4% vs DGC 31.7% p=0.09). Total no of blastocysts and top quality blastocysts

were significantly higher in MSS group than in DGC (3.9 vs 2.5 p<0.01 and 1.6 vs 0.8 p<0.001 respectively).
Limitations, reasons for caution: Retrospective design, small sample size, lack of proper randomization and power analysis are the main limitations.
Wider implications of the findings: Offering PGT-A to couples with unexplained repeated IVF failures and high SDF seems feasible. MSS for such cases improves embryonic division process as improved blastulation rates were documented. However, euploidy rates were not improved in MSS group revealing that other factors influence comprehensive chromosomal status of an embryo.
Trial registration number: not applicable

P-067 Utility of evaluating semen samples from adolescents with Klinefelter Syndrome for cryopreservation: A multi-institution evaluation

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Study question: Should physicians continue to evaluate semen analysis from adolescents with Klinefelter Syndrome for fertility preservation?
Summary answer: In the largest multi-institutional retrospective database to-date for this patient population, no sperm was found in ejaculate for cryopreservation amongst adolescent males with Klinefelter Syndrome.
What is known already: Klinefelter Syndrome is the most common genetic condition leading to male infertility and non-obstructive azoospermia. The condition causes decreased testicular growth, leading to lower production of testosterone and resulting deficiencies in secondary sexual characteristics. While testosterone therapy may be required for hypogonadism, there may be impact on future fertility potential. Current practice is to have KS adolescent patients provide semen analyses to identify potential sperm for cryopreservation. While the incidence is low, current epidemiological studies have been with limited sample size.
Study design, size, duration: This was a retrospective study of all adolescent Klinefelter Syndrome patients seen at the male infertility clinics of two large academic institutions between the years of 2015 to 2020. Adolescence was defined as the ages of 10 – 19 years old, as per the World Health Organization.
Participants/materials, setting, methods: A total of 116 patients were identified for the retrospective study database. Demographic information including weight, height, comorbidities, concurrent medications were collected. Hormone levels such as FSH, LH, testosterone, and estrogen were included for 77 patients. Additionally, semen analyses were available for 49 patients. Main results and the role of chance: Of the 49 patients with semen analyses, only 3 patients had rare sperm in ejaculate not sufficient for cryopreservation while the remaining had azoospermia. The average ejaculate volume of the provided semen samples was 0.9 cc. The average serum total testosterone level of adolescent Klinefelter Syndrome patients was 236 ng/dL. As expected, gonadotropin levels were found to be elevated (mean: 18.47 IU/L for FSH and 9.12 IU/L for LH).
Limitations, reasons for caution: The main limitation for this study was the sample size.
Wider implications of the findings: The findings from the largest retrospective study of this patient population imply a need to revisit counseling regarding the need for semen analyses in adolescent Klinefelter Syndrome patients.
Trial registration number: not applicable

P-068 CatSper4 cation channel expression is low in male infertility and is affected by cryopreservation

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Study question: Is CatSper4 expression in sperm related to functional parameters and does cryopreservation affect CatSper4 expression?
Summary answer: In this study, it was aimed to investigate whether CatSper4 has a relationship with sperm parameters and is CatSper 4 affected by cryopreservation.
What is known already: CatSper membrane channels, known as cation channels, are thought to play an important role in the insufficiency of sperm physiology, acrosome reaction, and chemotaxis movement. There is no study on cation channel distribution in an infertile male patient. In addition, studies conducted in recent years have shown that cryopreservation techniques have negative effects on sperm DNA, but there is no analysis in the literature regarding the effects of cryopreservation on CatSper4 ion channel proteins.
Study design, size, duration: Samples of the patients who applied to the Andrology laboratory in the Medical Park Hospital IVF unit between March 1 and June 1 in 2020 were included in the study. Also, patients with no family history of no genetic anomalies , no varicocele and azoospermia were included.The study were divided into 4 groups in accordance with the male infertility guideline of the European Association of Urology as normozoospermic (control group), the asthenoteratozoospermia, teratozoospermia, and oligoastenotheratozoospermia.
Participants/materials, setting, methods: In this prospective study, semen analysis, DNA fragmentation, and CatSper 4 by IHC of control group patients with normospermia (n=40) and oligospermia(n=50), asthenospermia(n=40), and teratozoospermia(n=38) patients were compared and differences resulting from cryopreservation were evaluated by Wilcoxon signed Ranks Test.
Main results and the role of chance: It was observed that CatSper4 protein positivity was localized in the middle part of the sperm and it was statistically higher in the normozoospermic patient group compared to the other groups (p=0,01). When the positivity values of CatSper4 protein before and after freezing were compared in the groups, it was seen that the values decreased (p=0,001,p=0,01). Sperm DNA fragmentation was found to be lowest in normospermia and statistically significantly higher in other groups. Cryopreservation application increased DNA fragmentation in all groups (p<0,001 , p<0,01).
Limitations, reasons for caution: Unfortunately, embryo screening in patients with low CatSper4 expression is not available in the present study. Soon we plan to screen a broader clinical pregnancy series and present the IVF results associated with CatSper4.
Wider implications of the findings: Our study indicated that, CatSper4 expression is quite high in normospermia when compared with the other groups, particularly oligoasthenoteratozoospermia and asthenoteratozoospermia. There are almost no studies on this subject in the literature, and we think that it should be studied in larger patient groups and in unexplained infertile cases.
Trial registration number: not applicable

P-069 microfluidic sperm sorting vs density gradient to yield sperm with reduced DFI for patients undergoing IVF-ICSI

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Study question: To compare the effect of sperm preparation methods on the DFI of semen sample for patients undergoing ICSI.
Summary answer: On comparing the results, microfluidic sperm sorting yielded sperms with significantly less DFI as compared to density gradient method of sperm preparation.
What is known already: The DNA integrity of the sperm plays an important role to ensure formation of good quality embryos with increased potential of fertilization, growth and ultimately implantation.. Centrifugation has shown to add stress to the sperm and leading to DNA damage, therefore there is a need to develop techniques of sperm preparation which help in retrieving as many sperms with intact DNA from the unprocessed sample as possible. Microfludic is fluid dynamic based technique of sperm preparation. in this study, we evaluated if microfluidic sperm sorter can recover motile sperm with better DNA integrity compared to density gradient preparation method.
Study design, size, duration: Prospective randomized study conducted in 80 patients undergoing IVF-ICSI with normal semen parameters (based WHO criteria 2010). DFI was done using Sperm Chromatin Dispersion (SCD) test in split semen samples prepared by microfluidic sperm sorter and density gradient method. Sperm morphology and motility were also recorded and evaluated based on the WHO 2010 criteria.
Participants/materials, setting, methods: Semen parameters of the sample were assessed by microscopic examination. DFI of each unprocessed sample was carried out using SCD test, following that the sample was split and sperm preparation was done using microfluidic sperm sorter and density gradient. the recovered sperm were tested for DFI and the results were compared.

Main results and the role of chance: Mean DFI in unprocessed semen samples was 23%. the analysis of split semen samples post preparation showed that the DFI was significantly reduced with the use of microfluidic sperm sorter (mean DFI 0.6%) as compared to density gradient (mean DFI 9%).
Limitations, reasons for caution: A major limitation of the microfluidic sperm sorter is the use sperm concentration and motility of the semen sample. In oligospermic and asthenospermic samples, density gradient is the preferred method of preparation. Lack of data showing improvement in clinical outcomes with reduced DFI is also a major limitation.
Wider implications of the findings: Microfluidics has shown to significantly reduce the DFI of the semen sample, it requires no extra equipment and cost and is relatively easy to pick up. Density gradient method of sperm preparation continues to be the preferred method due to its versatility and recovery of good quality sperm.
Trial registration number: not applicable

P-070 Evaluation of SARS-CoV-2 in human semen and effect on total sperm number: A prospective observational study

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Study question: Is the SARS-CoV-2 virus present in human semen and what is the impact on semen parameters following an infection?
Summary answer: SARS-CoV-2 infection, though not detected in semen of recovered men, can affect TSN in ejaculate in the acute setting.
What is known already: Early epidemiological data has suggested that the primary mode of transmission is through respiratory droplets, but the presence of SARS-CoV-2 has been identified in other bodily fluids such as feces, urine, and semen.
Study design, size, duration: We prospectively recruited thirty men diagnosed with acute SARS-CoV-2 infection using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) of pharyngeal swab specimens. Thirty semen samples from recovered men were obtained 11-64 days after testing positive for SAR-CoV-2 infection. The median duration between positive SAR-CoV-2 test and semen collection was 37 days (IQR=23).
Participants/materials, setting, methods: Semen samples were collected from each individual using mailed kits. Follow-up semen samples were done with mailed kits or in-person in office setting. Semen analysis and PCR was performed after samples were received.
Main results and the role of chance: The median total sperm number (TSN) in ejaculate was 12.5 million (IQR=53.1). When compared with age-matched SARS-CoV-2(-) men, TSN was lower among SARS-CoV-2(+) men (p=0.0024). Five men completed a follow-up sperm analysis (median 3 months) and had a median TSN of 18 million (IQR=21.6). No RNA was detected by means of RT-PCR in the semen in 16 samples tested.
Limitations, reasons for caution: First, most of the semen samples came from non-severe men of whom were in the recovery stage and lacked symptoms. Additionally, our sample size was relatively small and overnight mail-in semen analysis kits were used during the acute phase of infection to minimize contact with positive subjects.
Wider implications of the findings: Our findings suggest extremely low risk of viral transmission during sexual contact and assisted reproductive techniques, although further data need to be obtained. The impact on TSC in recovered men from SARS-CoV-2 infection is concerning, nevertheless long-term follow-up of these men is critical to determine the nadir of TSC.
Trial registration number: 20200401

P-071 Human Papillomavirus Infection and impact on men fertility

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Study question: Is there any correlation between men infertility and HPV infection and its impairment on sperm quality?
Summary answer: There is a significantly higher prevalence of high-risk HPV in infertile men than fertile men. HPV infection does not impair sperm quality.
What is known already: Many factors may cause the infertility in males and females. Human papillomavirus are the most frequently sexually transmitted DNA viruses and etiological agents of cervical cancers. There is association between HPV infection in females and adverse pregnancy outcomes such as spontaneous abortion and spontaneous preterm delivery. Previous studies have reported the detection of HPV DNA in semen and in different sites of the male reproductive tract, such as glans penis and scrotum. Recent reports suggested that HPV may affect sperm parameters and lead to male infertility. The impact of HPV infection upon male fertility abnormality has received far less attention.
Study design, size, duration: In this study case control study we examined 38 fertile and 36 men from infertle couples.
Participants/materials, setting, methods: we examined the swabs of the entire penile surface and semen samples for HPV detection and genotyping from 38 fertile men and 36 from men from infertile couples.HPV were detected with PCR method. Sperm was also examined for its motility, sperm quantity and morphology .
Main results and the role of chance: Among 36 confirmed infertile males, only 8 (22.22 %) cases were tested positive for HPV of semen samples and 2 among fertile men were HPV-positive (5.26%) of semen samples. Among infertile males 14 (38.88%) had HPV positive penile swabs, and only 1 (2,63%) had positive HPV swab among fertile men. The most prevalent HPV types in the male external genitalia were HPV-16. The most prevalent HPV types in semen were HPV-53. This data revealed a significant association between high-risk HPV and male infertility (P=0.03). Sperm quality (morphology and motility) did not differ significantly between men with seminal HPV infection and uninfected men.
Limitations, reasons for caution: There were some limitations in the study such as differences in age. sample sizes and the number of HPV genotypes detected.
Wider implications of the findings: We need larger studies and more further investigations to confirm the impact of HPV on male infertility.
Trial registration number: 2

P-072 Pregnancy rate in male factor infertility with oligoastheneteratozoospermia - evaluation of Letrozole and Coenzyme Q10 supplementation on sperm parameter

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Study question: Male infertility due to idiopathic oligoasthenoteratozoospermia- Does combining Letrozole as antiestrogenic with Coenzyme Q10 as antioxidant give better pregnancy rate ?
Summary answer: Combination of Co enzyme Q10 with Letrozole can significantly improve semen parameters and outcome of clinical pregnancy rate in idiopathic oligoasthenoteratozoospermic patients.
What is known already: Elevated levels of reactive oxygen species(ROS) are a major cause of idiopathic male factor infertility which results in sperm membrane lipid peroxidation, DNA damage and apoptosis leading to decrease sperm viability and motility. Antioxidant like Coenzyme Q10 have been used empiricallyin the treatment of oligoasthenozoospermia based on its ability to reverse oxidative stress and sperm dysfunction. Aromatase inhibitor like Letrozolehave been used in idiopathic male infertility by reducing estrogenic effect on spermatogenesis and reducing feedback inhibition of hypothalamopituatarygonadal axis. Thus a therapeutic strategy would need to use supplements to increase sperm energy metabilism, minimise free radical damage.
Study design, size, duration:
Study design: prospective comperative clinical study
Primary purpose: treatmenr
Size: 60 infertile male attending OPD of SHRISTI HEALTHCARE diagnosed as idiopathic oligoasthenoteratozoospermia
Duration: from March2018 to February 2020
Primary outcome: improvement in sperm count, motility and morphology after treatment
Secondary outcome: clinical pregnancy rate and live birth rate.
Participants/materials, setting, methods: Exclusion criteria: Smoker, drug and alcohol abuse, medical treatment with gonadotropin and steroids, varicocele.60 patients were randomisedinto 3 groups. Gr A(N=20) received Letrozole 2.5mg/day + Co enzyme Q10 300mg/day for 3 months, Gr B(N=20) received Letrozole 2.5mg/day for 3 months, and Gr C(N=20) received Coenzyme Q10

300mg/day for 3 months. History taking, general examination, semen analysis, sr.FSH,LH, Testesteron, E2 and scrotal duplex were done for all patients.
Main results and the role of chance: After treatment, Gr A as compared to Gr B and C showed significant imprivement in all 3 parameters of semen eg sperm count(3.15±3.38 - 20.9±2.11, p<0.001), sperm motility(5.25±3.25 - 42.85±3.30, p<0.001), sperm morphology(2.26±7.81 - 25.89±7.05, p<0.001). Improvement in sperm count and morphology was seen in Gr B(Letrozole gr) but not in sperm motility whereas Gr C (Co enzyme Q10 gr)showed significant improvement in sperm motility and morphology but not in sperm count. 10 pregnancies occured during follow up period of 1 yr. Clinical pregnancy rate was 30%in Gr A(6/20), 5% in Gr B(1/20), AND 15% in Gr C(3/20). Live birth rate was 83% in Gr A(5/6), 33.3% inGr C(1/3) whereas sponteneous abortion occured in Gr B pregnancy.
Limitations, reasons for caution: Limitation of my study was the small sample sizewhich could have some bias in outcome. I did not evaluate DNA fragmentation and level of ROS. Latest evidences report that evaluating ROS can be a diagnostic tool in predictingthe best responder to supplementation.
Wider implications of the findings: Majority of studies had investigated the effect of antioxidant and aromatase inhibitor on semen parameter but few concluded their effect on live birth rate. Assisted reproductive techniques are expensive and not universally available, so any pharmacological agent with satisfactory effectiveness should be considered as 1st line treatment of oligoasthenoteratozoospermia.
Trial registration number: NOT APPLICABLE

P-073 High-intensity interval training modulates the effects of hypertension on inflammatory mediators in testis in adult spontaneously hypertensive rats

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Study question: Is the high-intensity interval training (HIIT) able to prevent the increase of the inflammatory proteins in the testis of spontaneously hypertensive rats (SHR)?
Summary answer: HIIT for 8 weeks inhibits the increase of the tumor necrosis factor alpha (TNFα) and the interleukin 6 (IL6) in the testis of SHR.
What is known already: Hypertension increases the inflammatory cytokines of the cardiovascular system, causing damage in the microcirculation and in the testes. Hypertension is a cause of low fertility in men and exercises are indicated to decrease blood pressure and improve overall health. HIIT is characterized by short periods of exercise, with an intensity equal to or greater than the anaerobic threshold, separated by recovery periods. HIIT can be indicated for cardiac patients, as this type of training improves the cardiac autonomic nervous system and lipemic control in both hypertensive and normotensive individuals.
Study design, size, duration: Male Wistar-Kyoto rats without hypertension and SHRs were divided into 3 groups (n=4): WKY (Wistar-Kyoto); SHR; and SHR-HIIT (SHRs that performed HIIT). The HIIT was realized in treadmill for 5 days/week for 8 weeks. HIIT started with 5 times of 4 minutes of 100% of the maximum exhaust speed, with active rest intervals of 3 minutes at 60%. There was an increase of once every week until reaching 7 times each session.
Participants/materials, setting, methods: Samples from testicles were used for immunostainig IL6 and TNFα. After the blocking, the sections were subjected to reaction of specific antibodies IL6 (1:50, E-4, sc-28343) and TNFα (1:50, 52B83, sc-257) at 4°C overnight, and with secondary antibodies m-IgGK (1:200, IgG-HRP, sc-516) at room temperature for 2 hours. Diaminobenzidine (1:50) was used against Harris stained with Hematoxylin and evaluated in the photomicroscope. Data were analyzed by One-Way ANOVA followed by Tukey post test (P<0.05).
Main results and the role of chance: The immunostainig of IL6 was higher in SHR (10.23 ± 0.47) than in SHR-HIIT (8.69 ± .44) group (P=0,0237). From the same perspective, immunoexpression of TNFα was higher in SHR (10.10 ± 0.42) than WKY (8.24 ± 0.24) and SHR-HIIT (7.82 ± 0.39) groups (P=0.0018).
Limitations, reasons for caution: As a limitation of the study, we have no measurement of fertility parameters to affirm that the HIIT improve the fertility because of the reduction of inflammatory mediators.
Wider implications of the findings: The hypertension drugs have a negative effect on fertility. HIIT can be suggested as a treatment for hypertension as an alternative to medication, since HIIT can inhibit the increase of the inflammatory mediators in testis and its consequences to the reproduction. Financial support by São Paulo Research foundation, FAPESP (2018/22682-0).
Trial registration number: 2018/22682-0

P-074 Chromatin Maturity Index (CMI) in unfixed and live spermatozoa and Aniline Blue (AB) stained as an additional evaluation parameter in idiopathic male infertility

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Study question: To investigate whether idiopathic male infertility may be due to the presence of histones in motile spermatozoa using a modified AB staining protocol.
Summary answer: No correlation between CMI in live motile spermatozoa, DNA Fragmentation Index (DFI) and other conventional seminal parameters were found in male infertile patients.
What is known already: The AB stain discriminates between lysine-rich histones and arginine/cysteine-rich protamines. Transition from histones to protamines during spermatogenesis remodels chromatin packaging and abnormalities in the substitution of those proteins maybe interfere with seminal parameters and affect male infertility. The correlation between CMI and seminal parameters is known, but little is knowledge about live and motile spermatozoa associated to CMI because literature report only spermatozoa fixation before staining. Sperm chromatin carries half of the genomic material to offspring. Spermatozoa nuclear status is crucial for balanced transmission to future generations, and histones modifications are directly involved in epigenetic mutations.
Study design, size, duration: Retrospective observational study of 77 men underwent to standard semen analysis, including the evaluation of CMI and DFI, enrolled from January to December 2020. Mean age of the men was 36.63±8.26 years old, sperm concentration 46.69±37.23 mill/mL, linear progressive motility 39.35±15.31%, normal morphology 6.42±3.40%, DFI 25.91±10.29%. 200 spermatozoa for evaluation of CMI and 300 for DFI were analyzed respectively.
Participants/materials, setting, methods: Semen samples of 77 patients were collected and analyzed according to 5th edition of WHO guidelines (2010) for examination of human semen. For the evaluation of CMI we performed a new modified protocol for AB stain directly in live spermatozoa. Dilution 1:1 fresh semen and Aniline Blue colorant were mixed and placed on a slide and examined in bright field microscopy x1000 magnification. DFI was evaluated using Sperm Chromatin Dispersion (SCD) test.
Main results and the role of chance: Of all spermatozoa analyzed, 82.58±29.98% were white, 17.17±17.21% were pale blue, and 28.53±21.09% were dark blue. By our modified protocol, directly in live spermatozoa, we correlated AB staining with motility and , surprisingly, all motile spermatozoa

observed were not stained (white), while pale or dark blue spermatozoa resulted always immotile. For this reason, we have considered pale blue spermatozoa as AB positive, in disagreement with some authors. So, maybe, we should reconsider pale blue stained spermatozoa as abnormal. We also observed AB negative spermatozoa with morphological head, neck and tail defects, underlining the independence of these two parameters: nuclear status and morphology. We have observed no statistically significant differences between conventional semen parameters, DFI and CMI, so nuclear analysis seems to be independent parameters. The statistical analysis was performed by Matlab statistical toolbox version 2008 (MathWorks, Natick, MA, USA) for Windows at 32 bit; finally all tests with *p-value (p)* < 0.05 were considered significant. Attention should be paid to the evaluation of CMI not only in astenozoospermic patients, where a lower CMI is known, but also in normozoospermic infertile patients.

Limitations, reasons for caution: This is a preliminary observational study on a small number of normozoospermic or mild asthenozoospermic patients. The study should be considered as a pilot study. Future studies with higher number of samples are necessary in order to confirm the results obtained.

Wider implications of the findings: This is the first study that reports AB staining on unfixed live spermatozoa with a modified protocol. Our study underlines the necessity of classify pale blue spermatozoa as AB positive. Further investigations are necessary. This is a starting point for future analysis to be carried out under the project EcoFoodFertility.

Trial registration number: not applicable

P-075 HAART exacerbates anti-Koch-induced reproductive toxicity via suppression of androgen and down-regulation of cGMP signaling

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Study question: Will highly active antiretroviral drugs (HAART) and antikochs impair reproductive function when used singly and concurrently?

Summary answer: HAART exacerbates antikoch-induced reproductive toxicity by stimulating testicular and penile oxido-inflammatory response. This was associated with suppression of androgen and down-regulation of cGMP signaling.

What is known already: Although the advent of HAART and antikochs has significantly improved the clinical status, life expectancy and quality of life of patients with HIV/tuberculosis, these drugs are with shortcomings. Studies have reported that HAART induces testicular toxicity and impairs sperm quality. Similarly, antikochs has been shown to trigger oxidative testicular and sperm damage. Available data have implicated HAART and antikoch in the pathogenesis of male infertility via oxidative stress-mediated mechanism. However, no study has reported the impact of the concurrent administration of both HAART and antikochs as seen in patients with TB/HIV co-infection on testicular function, sexual behaviour and fertility outcome.

Study design, size, duration: This is a prospective experimental study using animal model. Forty sexually mature inbred male Wistar rats of comparable age were used for the study. The study lasted 8 weeks.

Participants/materials, setting, methods: Animals were acclimatized for two weeks after which they were randomly allotted into four groups (n=10). The control rats 0.5mL of distilled water as vehicle, anti-Koch-treated rats received a cocktail of anti-tuberculosis drugs (Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol), HAART-treated animals received a cock-tail of antiretroviral drugs (Efavirenz, Lamivudine, and Tenofovir), while the HAART+antikochs-treated rats received treatment as HAART-treated as well as antikoch-treated. The doses of drugs used were the Human Equivalent doses for rats.

Main results and the role of chance: HAART exaggerated antikoch-induced increase in testicular lactate dehydrogenase activity, concentrations of lactate and uric acid, and reduced testicular sorbitol dehydrogenase activity. Furthermore, HAART worsens antikoch-induced decline in the activities of testicular and penile superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase, as well as glutathione concentration, but increased malondialdehyde levels in testicular and penile tissues, as well as penile and testicular DNA fragmentation. Similarly, HAART aggravates antikoch-driven reduction in penile cGMP, circulatory and testicular testosterone, serum prolactin, LH and FSH, impaired sperm quality, sexual behaviour, and fertility outcome.

Limitations, reasons for caution: This is a prospective study using animal model; hence findings should be extrapolated to human with care. Human studies are thus recommended.

Wider implications of the findings: This study demonstrates for the first time the impact of HAART and antikoch, when used singly or in combination, on sexual behaviour, sperm quality and penile and testicular integrity. The findings add to the available literature by providing the molecular mechanism through which HAART and/or antikoch possibly impair reproductive function.

Trial registration number: N/A

P-076 Probability of sperm retrieval in azoospermic patients and mRNA expression profile of JMJD1A, TNP2 and PRM2 : in a subset of karachi population

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Study question: To access successfulness of sperm retrieval by evaluating the mRNA expression profile of JMJD1A, TNP1, TNP2, PRM1 and PRM2 in patients undergoing surgical sperm retrieval procedure.

Summary answer: Probability of sperm retrieval in azoospermia is decreased when mRNA expression profile of JMJD1A TNP2 and PRM2 in testicular tissue is decreased.

What is known already: Studies have been done on expression of JMJD1A in non-obstructive azoospermic patients in other part of the world with smaller sample size but this is the first study in Pakistan with larger number of patients. Study design, size, duration: Crossectional study, 100 azoospermic patients coming for purpose of sperm retrieval by TESE or micro-TESE in Australian Concept Infertility Medical Center, Karachi,from March, 2018 to December, 2019

Participants/materials, setting, methods: All recruited azoospermic patients were evaluated by history, physical examination, and hormonal assessment. RNA was extracted by pureLink RNA Micro kit and mRNA expression of the JMJD1A, TNP1, TNP2, PRM1 and PRM2 genes was determined using innu-SCRIPT One Step RT_qPCR SyGreen kit. For quantitative variables independent t test and for categorical variables chi-square/ Fisher Exact test was used. Unadjusted and adjusted prevalence ratio were reported by using cox regression algorithm. Main results and the role of chance: The patients were categorized into (i) Group-I: Patients with successful sperm retrieval n= 42, (ii) Group-II: Patients with unsuccessful sperm retrieval n= 58. The patients were categorized into (i) Group-I: Patients with successful sperm retrieval n= 42, (ii) Group-II: Patients with unsuccessful sperm retrieval n= 58. Azoospermic men in the successful sperm retrieval group had significantly decreased expression of JMJD1A (P<0.001), TNP2(P<0.001), and PRM2 (P 0.008). In addition to this regarding hormonal parameters: FSH (P 0.004), LH(P<0.001), TSH(P<.011) were significantly different in azoospermic men with successful and unsuccessful sperm retrieval. In multivariate analysis, after adjusting for the other covariates, a significant association was found between JMJD1A, TNP2, PRM2 and successful sperm retrieval (p-value <0.05).

Limitations, reasons for caution: It is unicentric and outcomes for fertilization were not assessed. Azoospermic patients from multi-centeres were difficult because of lack of facility of sperm retrieval procedures at these centers and it was difficult to follow the fertrilization outcome.

Wider implications of the findings: This will be useful for making the decision in azoospermic men to proceed for ICSI or not. In addition to this, the repetition of unnecessary surgical procedures can be avoided, as the azoospermic men often undergo number of rounds of ICSI, with the hope of becoming biological father.

Trial registration number: non-clinical trials

P-077 Performance of the postwash total motile sperm count as a predictor of pregnancy at the time of intrauterine insemination

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Study question: Is the performance of the postwash total motile sperm count a predictor of pregnancy at the time of intrauterine insemination?
Summary answer: The number of motile spermatozoa inseminated (NMSI) in IUI can be used to define clear range of pre /postwash sperm parameters.
What is known already: There is no consensus about the optimal number of motile spermatozoa inseminated (NMSI) required for a reasonable chance of pregnancy after IUI. A meta-analysis of 16 studies assessing NMSI and IUI outcomes, concluded that at cut-off levels between 0.8 and 5 million, defined as the ability to predict failure to become pregnant. The purpose of this study is to determine the range of NMSI as a predictor of success in IUI.
Study design, size, duration: This prospective study includes 60 patients who underwent semen analysis at an academic infertility centre (SRIHER) during the month of December 2020 and January 2021.
Participants/materials, setting, methods: A total of 60 infertile couples who underwent IUI at our academic centre were enrolled in our study. A detailed history and infertility work up was done before proceeding for IUI, as per the department protocol. The semen was prepared by discontinuous 2 layered density gradient method. The results were analyzed by patient factors including age, BMI, semen parameters, NMSI. The NMSI were divided into 4 groups: A (<1), B (1-4), C (5-9), D (>10).
Main results and the role of chance: The mean age of the infertile couples who underwent IUI was (28.2 ± 3.8) in females and (31.8 ± 3.8) in males respectively. The sperm parameters such as concentration (21.8 ± 14.8), motility (53.15 ± 13.22), morphology (2.43 ± 1.33) respectively. When the NMSI was group C (5-9 x 10^6) the pregnancy rate was 38.5 % whereas the pregnancy rate was 37.5% in group D (>10 x 10^6). In the other sub groups such as group A(<1 x 10^6) and group C (1-4 x 10^6) the pregnancy rate was 14.2% and 12.5 % respectively.
Limitations, reasons for caution: Infertile men with Azoospermia, Men with Retrograde ejaculation, Testicular samples, Epididymal samples, Infected samples. All of the above samples were avoided. It cannot be used for counselling during the initial infertility workup, but only during/after the IUI procedure.
Wider implications of the findings: The results suggest that NMSI can be a predictor of success in IUI in patients who are < 30 years of age & ≥35 years, NMSI does not appear to be a useful. The effect of NMSI on pregnancy rate needs to evaluated on a larger scale .
Trial registration number: not applicable

P-078 Prognostic factors for male fertility recovery after microsurgical varicocelectomy

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Study question: What clinical and laboratory parameters are reliable predictors of spontaneous pregnancy (SP) after microsurgical varicocelectomy in men from infertile couples?
Summary answer: Predictors of SP after microsurgical varicocele repair are the male age, baseline total sperm motility, and postoperative increase level of TPMSC.
What is known already: Varicocele is the most common correctable cause of male subfertility. According to the recent meta-analyses and studies, microsurgical varicocelectomy is the "golden standard" method for varicocele repair. However, it is still unclear why at least one-third of subfertile men do not experience improvement in semen parameters and more than half of them do not report fertility recovery after varicocelectomy. There is no consensus so far on the factors affecting the efficacy of varicocele repair in men from infertile couples.
Study design, size, duration: This retrospective study comprises 93 men from infertile couples, with palpable varicocele, astheno-/oligozoospermia, and who underwent microsurgical subinguinal or inguinal varicocelectomy from September 2015 to May 2019.
Participants/materials, setting, methods: The changes in semen analysis were assessed (in 3-6 months after surgery) according to WHO-2010, spontaneous pregnancy (SP) rates after surgery also were considered. A stepwise discriminant analysis was performed to identify predictors of SP after varicocelectomy. An increase in TPMSC by at least 12.5 million was defined as a significant effect (SE) of varicocelectomy (reference values for the total number and progressive sperm motility according to WHO-2010: 39 million x 0.32 (32%) progressively motile).
Main results and the role of chance: Almost all semen parameters (except for semen volume) changed positively after surgery. Sperm concentration increased from 62 mln/ml (17-107) to 85 mln/ml (39-134) p<0.001, TPMSC increased by an average of 27 mln (2.8 times; p<0.001). SE was observed in 52% of cases (n = 48), a slight favorable effect in 21% (n = 20), and no effect in 27% (n = 25). 29 patients (31%) reported SP within a year after varicocele repair. 83% of patients (24 from 29) who reported pregnancy after varicocelectomy showed SE. According to the stepwise discriminant analysis, significant predictors of pregnancy after varicocelectomy were the male age (coefficient of the canonical discriminant function = -0.16), the initial total sperm motility (0.02), and the postoperative increase of TPMSC (0.01). Wilks' lambda was 0.67 and canonical correlation 0.57. The predictive ability of the prognostic model (discriminant function) with these three predictors was 84%, specificity 87%, and sensitivity 76%. The function real predictive accuracy for SP was 70% (21 correct out of 30 predicted).
Limitations, reasons for caution: The small sample size and the inability to obtain accurate data on the health condition of female partners were the main limitations of the study. Nevertheless, the findings are statistically significant, which suggests that they can be extrapolated to the general sample of subfertile men with clinical varicocele.
Wider implications of the findings: The proposed algorithm (function) for the prediction of SP showed satisfied predictive accuracy, and after its external validation can be recommended in 3-6 months after varicocele repair to decide whether it is advisable to expect an SP within a year or to include an infertile couple in ART programs immediately.
Trial registration number: Not applicable

P-079 A spontaneous LH peak before triggering for intrauterine insemination with donor sperm (IUI-D) is associated to lower live birth rates

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Study question: Are live birth rates after IUI with donor sperm (IUI-D) and controlled ovarian stimulation comparable between women with a spontaneous LH peak vs those without?
Summary answer: Biochemical, clinical, ongoing pregnancy rates and live birth rates were higher among women without an LH peak.
What is known already: It is common clinical practice to trigger ovulation in IUI cycles once specific criteria are met; if a natural LH surge appears, adjusting the IUI timing may become necessary. Pregnancy rates seem to be slightly better when IUI is scheduled in relation to the presence or absence of an LH peak in non-stimulated cycles. In IUI with stimulated cycles, however, there is no consensus in the medical literature regarding the best moment to program the IUI, due to different inclusion criteria, different IUI timing and definition of LH peak among studies.
Study design, size, duration: Retrospective cohort study of 9,657 IUI-D cycles performed between 2012 and 2019 in one fertility center. IUI-D without LH peak (n=6,679) versus IUI-D with LH peak (n=2,978) were compared. Differences in pregnancy outcomes between study groups were evaluated using a Pearson's Chi2 test. A p<0.05 was considered statistically significant.
Participants/materials, setting, methods: The definition used to define an LH peak is > 10UI/L in the last follicular control. In cases without an LH peak,

when at least one dominant follicle reached 17mm, ovulation was triggered with human chorionic gonadotropin in the following 24h, and IUI-D was performed 38h after triggering. In cases with an LH peak, ovulation was triggered the 6h following the detection, and IUI-D was also performed 38h later.
Main results and the role of chance: The women BMI and age were comparable between groups, with a mean±SD of 35.2±4.8 years old, and 24.3±4.7 for BMI. Other characteristics such as number of previous inseminations, type of stimulation drug, initial dose, total dose, stimulation length and number of follicles > 16mm in the last follicular control were also comparable. As expected, the LH level at the last follicular control was different between groups, with a mean of 5.1UI/L in the no-LH peak and 21.4IU/L in the LH peak group. The group without an LH peak had higher biochemical, clinical, ongoing and live birth rates compared to the group with LH peak: 27.7% vs. 20.7%; 19.5% vs. 15.5%; 17.7% vs. 13.7%; 16.3% vs. 12.6%, respectively (p-value<0.001).
Limitations, reasons for caution: The main limitation of the study is its retrospective nature. Also, a definition of LH peak based in absolute values was used; a definition based in relative values may lead to different results.
Wider implications of the findings: A definition of LH peak based on absolute numbers is imprecise, and the cut-off of 10UI/L does not allow a good scheduling for IUI. A LH peak based on relative values could improve the detection of patients starting ovulation and the accuracy in programming IUI.
Trial registration number: not applicable

P-080 Higher sperm DNA fragmentation reduces the proportion of good quality embryos at day 5 on IVF and ICSI cycles from unselected males

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Study question: Does sperm DNA fragmentation (SDF) reduce the ratio of good-quality embryos in day 3 (D3) and day 5 (D5) of embryonic development?
Summary answer: High sperm DNA fragmentation (SDF >15%) is associated with poor embryo quality at blastocyst-stage per cycle in unselected patients undergoing IVF and ICSI.
What is known already: It has been shown that the proportion of spermatozoa with DNA fragmentation is higher in infertile men than in semen from fertile men. However, the controversy regarding the impact that sperm genome damage can have on IVF or ICSI treatments is evident in the published literature. The effects of SDF would become evident after activation of the embryonic genome at 8-cell stage, compromising not only the quality of the embryos obtained, but also the reproductive outcomes, as reduced implantation rates, higher miscarriages rates and thus, a decreased chance of pregnancy.
Study design, size, duration: This multicentric observational retrospective study included 1339 couples who underwent 2759 IVF-ICSI cycles using autologous oocytes from January 2000 to March 2019. All men have an SDF test in their ejaculated spermatozoa by TUNEL assay (Terminal deoxynucleotidyl transferase dUTP nick end labeling). The subjects were divided into two groups according to their sperm DNA integrity: low (≤15%) (n=2287 cycles) or high (>15%) (n=472) SDF.
Participants/materials, setting, methods: Embryo quality was assessed complying morphological standards at cleavage-stage on D3 and at blastocyst-stage on D5 (inner cell mass (ICM) and trophectoderm (TE) grade (A, B, C or D)) in according to ASEBIR's embryo selection criteria, being embryos of good quality those categorized as A+B. The outcomes were calculated in relation to the total number of zygotes obtained. The results were compared by Student t test; p value <0.05 was considered significant.
Main results and the role of chance: The SDF average of the low group was 5.8% (95% CI 5.6-5.9) whereas in high group was 23.7% (95% CI 23.0-24.4). The female age was equal, 37.1 years (95%CI 37.0-37.2) and 37.1 years (95% CI 36.8-37.4) respectively. A total of 9796 embryos were evaluated. The optimal cleavage-stage embryo rate per cycle was 25.0% (95% CI 21.7-28.3) (8.0 average cells number, 1.5 embryo fragmentation average, symmetry 1, mononucleated cells) versus 26.7% (95 %CI 19.1-34.2) (7.9 average cells number, 1.8 embryo fragmentation average, symmetry 1, mononucleated cells) when comparing between groups (p<0.001). Blastocyst-stage arrival rate (number of embryos at D5) per cycle was 55.8% (95% CI 54.3-57.2) in ≤15% SDF group (embryo quality score was ICM A:12.1%, B:69.5%, C:8.8%, D:4.5%; TE A: 7.5%, B:42.2%, C:42.2%, D:8.1%) and 55.9% (95% CI 52.8-59.1) in the >15% SDF group (ICM A:12.0%, B:68.7%, C:10.6%, D: 5.2%; TE A:9.1%, B:44.8%, C:37.8%, D:8.3%) (p<0.001). The good quality blastocyst rate per cycle was significantly higher in the group with SDF ≤15%, 27.7% (95%CI 26.5-29.0) versus SDF >15% (27.4% (95%CI 24.6-30.2)). Of the total number of blastocysts, the proportion of A+B blastocyst was 60.5% (95% CI 58.3-62.7) and 64.2% (95% CI 59.2-69.2) (p<0.001), respectively.
Limitations, reasons for caution: The retrospective and multicenter nature of this study leads to uncontrolled biases derived from the clinical practice. Although the results were not adjusted for female's age, it was not statistically different between groups. Embryo morphology evaluation was performed by senior embryologists, it still remains a subjective evaluation, though.
Wider implications of the findings: In this study, a higher amount of data was compiled so that a large number of embryos were analyzed. The DNA integrity of the sperm may be an important consideration when poor quality embryos were obtained in the previous cycle when deciding on the next clinical strategy to apply.
Trial registration number: NA

P-081 Microfluidic sorting does not improve clinical outcomes compared to magnetic activated cell sorting (MACS) in Assisted Reproduction

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Study question: Does microfluidic sorting improve clinical outcomes over magnetic activated cell sorting (MACS) in ovum donation cycles?
Summary answer: Performing microfluidic sorting does not seem to improve clinical outcomes compared to MACS in ovum donation cycles.
What is known already: Novel sperm selection techniques, such as magnetic activated cell sorting (MACS), have been described as useful procedures to increase reproductive outcomes when male factor is present. Because of centrifugation steps associated to swim-up or density-gradient can induce sperm DNA fragmentation, microfluidic sperm sorters are being used to isolate motile human spermatozoa based on fluid dynamics and avoiding sample manipulation. This new technology has been shown to reduce the level of sperm DNA damage, especially double strand breaks, but an improvement of clinical outcome by using this technique remain unclear.
Study design, size, duration: Prospective and observational study to evaluate the efficacy of a sperm sorting technique based on microfluidic technology versus a technique based on the removal of apoptotic spermatozoa by MACS. The study was performed between May 2019 to January 2021 in IVI Madrid and IVI Sevilla. All men attending for an ovum donation cycle during the aforementioned study period were included. The exclusion criteria were sperm concentration <5 mill/mL and <15% of progressive motile spermatozoa.
Participants/materials, setting, methods: Seminal samples from couples participating in the study were divided into two aliquots; each of them was processed according to one of the study methods. Subsequently, each of the processed sperm samples was used to microinject half of the oocytes obtained during oocyte retrieval. In all case, a single-embryo transfer was performed. Variables were expressed as mean values and standard deviations. Statistical analysis was performed by ANOVA and Chi-squared where applicable; significance established under 0.05.
Main results and the role of chance: We included 48 couples in the study; of these, n=31 transferred an embryo derived from a MACS processed sperm

sample, while n=17 received an embryo from the microfluidic one. Groups were homogeneous in terms of number of transferred embryos and frozen embryos, usable blastocyst rate and fertilization rate; results were as follows for MACS and microfluidic processing respectively: number of transferred embryos (1.0±0.0 vs. 1.0±0.0, p=0.978); number of frozen embryos (1.6±0.5 vs. 1.4±0.7 p=0.168); usable blastocyst rate (40.7% vs. 43.1%, p=0.384); and fertilization rate (80.4% vs. 75.3%, p=0.075). However, according clinical outcomes, we observed significant differences in implantation rate (74.2% vs. 58.8%, p<0.001) and in clinical pregnancy rate (74.2% vs. 58.8%, p<0.001); finally, the miscarriage rate was similar between the two groups of study (6.4% vs. 5.8%, p=0.876).
Limitations, reasons for caution: This study has not considered the indication in male factor couples due to a high degree of double-strand DNA fragmentation. Therefore, more specific studies are required to determine in which patients, microfluidics sorter selection would significantly improve clinical outcomes.
Wider implications of the findings: In an unselected population**,** magnetic activated cell sorting significantly improves clinical outcomes compared to a microfluidic technique, so this latter method should not be recommended without a male factor indication associated with sperm DNA damage. The proposed microfluidic technology does not seem to offer a flow-free approach to select spermatozoa.
Trial registration number: NCT04061486

P-082 Effect of semen hyper viscosity (SHV) on blastocyst formation rate and implantation rate

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Study question: Does semen hyper viscosity effects blastocyst formation rate
Summary answer: Hyper viscosity of semen sample later results in poor blastocyst formation rate and lower implantation rate.
What is known already: Normal range of semen hyper viscosity ranges between 12-29%.Highly viscous semen samples impairs the physical and chemical characteristics of seminal fluid and due to which seminal oxidative damage increases which further increases the ROS and reduces the sperm motility there are some factors that can affect the seminal viscosity out of which one is Male accessory gland infection, Hypo function of prostate seminal vesicles and varicoceles. SHV create hindrance in semen preparation.
Study design, size, duration: Retrospective study was conducted from June 2019 to Oct 2020 at IVF unit IKDRC hospital.
Participants/materials, setting, methods: 142 patients were enrolled from June 2019 to Oct 2020 in IVF unit IKDRC hospital and divided into two groups. Group A (n=83) patients with hyper semen viscosity and Group B (n =69) patients with normal semen viscosity, inclusion and exclusion criteria's were same for both the groups, only patient with normozoospermia were taken. Semen analysis was done by using WHO manual 2010.
Main results and the role of chance: In group A with hyper semen viscosity fertilization rate was (49.2% vs. 70% p= <0.001) vs in group B with normal semen viscosity which is significantly higher in group B, Blastocyst formation rate (18.4% vs 35% p=<0.01) and implantation rate (9.4% vs 20% p=<0.005) both are significantly higher in group B . Which implies fertilization rate , blastocyst formation rate and implantation rate is significantly lower in patients with semen hyper viscosity.
Limitations, reasons for caution: Larger randomized control studies are needed to strengthen these results.
Wider implications of the findings: Our study demonstrates that patients having higher semen viscosity have poor blastocyst formation rate and implantation rate due to oxidative stress.
Trial registration number: not applicable

P-083 Analysis of chromosomal segregation and interchromosomal effects (ICE) in sperms from balanced translocation carriers using fluorescence in situ hybridization (FISH) after sperm selector separation

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Study question: Influence of sperm selector separation of sperms on their translocation load, segregation pattern, motility and occurrence of interchromosomal effects
Summary answer: Sperm selector separation led to reduction of the translocation load, shift in segregation pattern and lower rates of interchromosomal effects within sperm samples
What is known already: Balanced translocations in men are known to be one of the main causes of reproductive failure. The segregation pattern in sperms is determined by the distribution of the chromosomes during meiosis. Interchromosomal effects can also influence the distribution of chromosomes that are not involved in the translocation. The sperm selector used consists of two concentric chambers, which are overlaid by a U-ring and a cover glass. Motile sperms migrate from the native ejaculate in the medium filled inner chamber by using a capillary bridge created by the U-ring. This avoids potential harmful centrifugation and allows accumulation of motile sperms.
Study design, size, duration: Twenty-one carriers of balanced translocations participated in the study. In addition, 15 patients were involved as control. All participants signed an informed consent (F-8-15). Samples of three patients did not meet the internal quality criteria and had to be excluded from analysis. The study started in 2015 and is still ongoing.
Participants/materials, setting, methods: Liquefied native ejaculate was processed with a sperm selector. Native ejaculate, non-migrated sperms from the outer chamber and migrated sperms from the inner chamber were transferred onto glass slides, fixed and underwent a decondensation treatment. For segregation analysis FISH translocation specific FISH probe mixes were used and tested on patient's blood. Interchromosomal effects were analysed with FISH probes for the chromosomes X, Y, 18 and 13, 21. Evaluation was done manually using fluorescence microscopy.
Main results and the role of chance: Segregation analysis was done for more than 25,000 sperms from men carrying a balanced translocation (18 patients with reciprocal and 3 patients with Robertsonian translocation). Separation via sperm selector led to a reduction in translocation load (native to separated approach 49,1±11,5% to 34,8±9,4% (P=<0,01), the rate depending on the specific translocation. There was also a shift in the segregation pattern, which seemed to be influenced by the specific translocation and the resulting steric alignment of the corresponding quadrivalent / trivalent. Additionally, more than 90,000 sperms from patients with balanced translocations were analysed for interchromosomal effects. Separation led to reduced maldistribution rate (native to separated approach 7,1±3,5% to 5±3,1%, P=<0,01) whereas the steric alignment of the corresponding quadrivalent / trivalent seems to influence the interchromosomal effect as well. For control, sperms from control patients were analysed regarding the chromosomes X, Y, 18 and 13, 21. In about 90,000 control sperms separation led to reduced maldistribution rate (native to separated approach 5,4±1,5% to 3,8±1,1%; P=<0,01).
Limitations, reasons for caution: The number of accumulated strand-break-free sperms depended on the motility and sperm count of the native ejaculate. Examinations are not reproducible, as each sample delivery is influenced by external circumstances
Wider implications of the findings: Sperm selector separation can be used before ART to reduce the translocation load and rate of maldistribution in sperms from carriers of balanced translocations. This could have a considerable impact on PGT results after trophectoderm biops.
Trial registration number: not applicable

P-084 Microfluidic Sperm Sorting (MFSS) technique versus Physiological Intracytoplasmic Sperm Injection (PICSI) technique in high DNA fragmentation index sperm samples

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Study question: To evaluate the effectiveness of using Microfluidic Sperm Sorting (MFSS) technique and Physiological Intracytoplasmic Sperm Injection (PICSI) technique in patient with high DNA fragmentation index (DFI) sperm samples.

Summary answer: Sperm selected by microfluidic sorting are associated with significant increase in day 3 grade A embryo development rate, clinical pregnancy rate over PICSI.

What is known already: DNA damage is unrecognisable in living sperm prior to insemination and an increased sperm DNA fragmentation index has been associated with lower fertilization rates, impaired embryo development and reduced pregnancy rates. Standard semen processing techniques are associated with centrifugation, which may induce reactive oxygen species and DNA damage. In strategies to minimize sperm DNA fragmentation, Physiological ICSI can relatively reduce sperm DNA fragmentation by 67.9% (Parmegiani et al., 2010) while new technique Microfluidic sperm sorter technique also demonstrate sperm selection with significantly reduced DNA damage.

Study design, size, duration: A prospective randomised study was conducted from 1st August 2019 to 31st December 2020. Two hundred patients were randomised by computer generated list and divided into 2 groups. Group A (n=100) , in which sperm were processed by microfluidic sperm sorter (MFSS) while in group B (n=100), sperm were selected by Physiological Intracytoplasmic Sperm Injection (PICSI) technique and morphologically normal motile sperm were injected by Intracytoplasmic sperm injection (ICSI) technique in all mature oocytes.

Participants/materials, setting, methods: The study period included all normozoospermic patients with high DNA fragmentation index (>25%) while oligospermic, asthenozoospermic samples, patients with poor ovarian reserve and advanced age were excluded from the study. All A grade embryos were vitrified and transferred in frozen embryo replacement cycle. Both groups were compared on the basis of fertilisation rate, day 3 grade A embryo development rate , clinical pregnancy rate and miscarriage rate.

Main results and the role of chance: Cycle characteristics (female age, length of stimulation, gonadotrophin dose, number of oocytes and number of transferred embryos) were similar in both groups. Between the 2 groups, There was a significant increase observed in day 3 grade A embryo development rate (60% vs. 42%, p-0.016) and clinical pregnancy rate (62% vs. 46%, p-0.049), while no statistical significant difference observed in fertilisation rate (82% vs. 78%, p-0.80) and miscarriage rate (12% vs. 11%, p- 1). Limitations, reasons for caution: Larger randomised control studies are needed to strengthen these results.

Wider implications of the findings: We have demonstrated that sperm sorted by microfluidic helps in selection of sperm with better DNA integrity over Physiological ICSI. Using it in routine practice can help in reducing the negative effect of reactive oxygen species and thus improve pregnancy rate and live birth rate.

Trial registration number: MCDH/2019/31

P-085 Sperm retrieval after microdissection testicular sperm extraction (micro-TESE) and pregnancy outcomes of intracytoplasmic sperm injection (ICSI) in men with Klinefelter syndrome

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Study question: What are the sperm retrieval rate and ICSI outcomes in azoospermic men with Klinefelter syndrome (KS)?

Summary answer: In men with KS, a sperm retrieval rate of 51.3% after the first attempt micro-TESE, and 4 live births after ICSI were observed. What is known already: Klinefelter syndrome (KS) is encountered in 10% of men with azoospermia. Micro-TESE is presently used to treat infertility for KS patients with nonobstructive azoospermia. The retrieved sperms can be used for ICSI. Study design, size, duration: From June 2019 to July 2020, 39 azoospermic patients with KS were examined for the presence of testicular spermatozoa. Participants/materials, setting, methods: Participants were recruited from couples attending the Andrology and Fertility Hospital of Hanoi Vietnam, for infertility treatment. Micro-TESE was performed to extract testicular tissue. After retrieval, ICSI was used with fresh sperm. Main results and the role of chance: The sperm retrieval rate of first attempt micro-TESE in KS men was 51.3% (20/39). Logistic regression analysis showed patient age did not affect the sperm retrieval rate of micro-TESE (OR 0.99, 95% CI 0.88 - 1.11). Similarly, no association was observed between serum FSH, LH, testosterone level, and testicular volume with the success of sperm retrieval. The fertilization rate after ISCI in patients with retrieved sperm was 60% (12/20). Clinical pregnancy and ongoing pregnancy rates were 50% (10/20) and 40% (8/20). There were 4 live births. No sufficient data were available to test the effect of clinical or biological parameters on ICSI outcomes.

Limitations, reasons for caution: Our data rely on a cohort of KS patients attending a single fertility clinic. The sample size did not allow regression analysis for any ICSI outcome.

Wider implications of the findings: Micro-TESE is helpful in retrieving sperm in azoospermia KS patients with sperm retrieval rate reaching 50%. ICSI following micro-TESE can lead to an ongoing pregnancy rate of 40% with some result in live births. The outcome of micro-TESE is independent of any clinical or biochemical parameters tested.

Trial registration number: Not applicable

P-086 High level of sperm DNA breaks in infertile men with varicocele: its association with sperm cells death, seminal oxidative stress, and spermatic parameters

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Study question: Our objectives were to determine the extent of nuclear sperm injury in varicocele patients with and without altered spermatic parameters and to investigate its relationship with apoptosis and oxidative stress.

Summary answer: Oxidative stress (OS) in the varicocele patients may play a role in the etiology of nuclear sperm DNA damage associated with apoptosis.

What is known already: Varicocele is associated with high level of DNA Breaks.

Study design, size, duration: Ejaculated sperm samples from 51 patients diagnosed with varicocele and 29 fertile men were examined. According to the guidelines, the patient's sperm samples were classified into varicocele with normal semen parameters (n = 11) and varicocele with abnormal semen parameters (n = 40).

Participants/materials, setting, methods: Sperm DNA breaks was assessed using terminal deoxynucleotidyl transferase dUTP nick end labeling assay. The proportion of both viable and dead spermatozoa with externalized phosphatidylserine was detected by the bivariate annexin V cy3/6-CFDA staining method. Seminal malondialdehyde (MDA) amounts and antioxidant enzymes activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured spectrophotometrically.

Main results and the role of chance: Sperm DNA Breaks, viable spermatozoa with externalized PS, and MDA levels were significantly higher in studied subgroups of patients with varicocele, either with normal or with abnormal semen parameters than controls. The seminal antioxidant enzymes activities were significantly reduced in both subgroups of patients with varicocele compared to the controls. The percentage of spermatozoa with fragmented DNA was positively correlated to the MDA level as well as the proportion of viable spermatozoa with externalized PS. However, the decreased seminal antioxidant status was negatively correlated with the increased proportion of sperm DNA fragmentation and apoptotic spermatozoa.

Limitations, reasons for caution: We suggest further comparative studies connecting the varicocele patients with and without altered spermatic parameters representing high level of DNA fragmentation with more apoptotic and oxidative stress markers.

Wider implications of the findings: This study reveals that impaired seminal antioxidant profile and increased seminal level of lipid peroxidation may be involved in the pathophysiological mechanisms of cell death-mediated DNA breaks in patients with varicocele.

Trial registration number: Not applicable

P-087 Paternal age clinical effect on cumulative live birth rates (CLBR) per inseminated oocyte, embryo transfer and embryo transferred in autologous IVF-ICSI cycles of infertile males

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Study question: Is reproductive success measured as CLBR per inseminated oocyte, per embryo transfer and per embryo transferred affected by paternal age in autologous IVF-ICSI cycles?
Summary answer: The number of embryo transfers and embryos transferred until live birth, but not the number of inseminated oocytes, were significantly different among the age groups.
What is known already: In recent years, there has been an increase of the average paternal age at which the first child is conceived. Therefore, there is a growing interest on the study of the impact of male age on the reproductive outcomes in assisted reproduction cycles (ART). Several studies have shown negative effects of advanced paternal age on semen parameters, embryo aneuploidy, miscarriage, male infertility,.... However, other studies have found no association between them. Hence, the impact of paternal age on reproductive outcomes still remains uncertain, leading to a need of more research on this topic, which this study tries to address.
Study design, size, duration: This retrospective observational multicentric cohort study has included autologous IVF-ICSI treatments (n=6295) performed to couples with etiology of male infertility (non-normozoospermic) in Spain IVIRMA clinics between January 2008 and March 2020 using patients' own sperm sample. Paternal age ranged from 20 to 75 years. The study population was categorized in 5 groups following the criterion of homogenizing the number of observations between groups: 20-34 (A), 34-37 (B), 37-39 (C), 39-42 (D) and 42-75 (E) years.
Participants/materials, setting, methods: Considering that male age could be a factor affecting reproductive outcomes, we evaluated men with different age that performed an autologous IVF-ICSI treatment with their own semen, etiology of male infertility and known age. Data was exported in order to obtain the clinical database and Kaplan-Meier was used for data analysis. P<0.05 was considered statistically significant. We measured reproductive success by CLBR per embryo transfer, per embryo transferred and per inseminated oocytes until live birth.
Main results and the role of chance: This study considered approximately 2976 patients and 4385 embryo transfers. The CLBR per inseminated oocyte showed no significant difference between the study groups: A (6.43%, 48.24%, 81.38%), B (5.74%, 52.14%, 82.87%), C (6.14%, 49.83%, 83.69%), D (5.89%, 53.60%, 81.16%) and E (6.61%, 47, 52%, 77.85%) for 4, 13 and 21/22 inseminated oocytes, respectively. In terms of CLBR per embryo transfer, the results obtained for each of the age groups were: A (31.81%, 71.89%, 87.63%), B (28.89%, 67.87%, 82.63%), C (27.10%, 68.87%, 88.17%), D (23.45%, 64.63%, 100.00%) and E (22.88%, 55.48%, 63.31%) for 1, 4 and 7 embryo transfers, respectively. There were statistically significant differences in the CLBR per embryo transfer between the studied age groups (p<0.0001). CLBR per embryo transferred for each age group was as follows: A (10.85%, 60.53%, 80.88%), B (9.34%, 59.75%, 78.23%), C (11.89%, 57.63%, 74.97%), D (10.25%, 52.71%, 77.76%) and E (11.71%, 51.50%, 71.51%) for 1, 4 and 7 embryos transferred, respectively. As in the case before, there were statistically significant differences in the CLBR per embryo transferred between the age groups (p<0.05). The findings presented highlight that the increase in paternal age could be affecting the reproductive outcomes in IVF-ICSI cycles using autologous oocytes.
Limitations, reasons for caution: The retrospective nature of this study leads to biases derived from the clinical practice and to the presence of missing data (limiting sample size). Moreover, this study considered autologous cycles, therefore the results were not adjusted for female factors.
Wider implications of the findings: Our study showed that reproductive outcomes measured by CLBR per embryo transfer and embryo transferred until live birth were significantly different between the paternal age groups in autologous IVF-ICSI cycles of infertile males. Hence, paternal age could be affecting reproductive outcomes and it should be considered for improving infertility counselling.
Trial registration number: NA

P-088 Sexual functioning is impaired in cancer survivors after cancer therapy

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Study question: Is impaired sexual functioning correlated to sperm quality in cancer survivors?
Summary answer: Erectile dysfunction affects 25.0% of cancer survivors, independent of sperm quality. 22.9% of patients show symptoms consistent with a reduced testosterone level.
What is known already: Gonadotoxic treatment in male cancer patients can end up in reversible or permanent impaired spermatogenesis, testosterone insufficiency, and sexual dysfunction.
Study design, size, duration: In this prospective single-center study, sexual functioning was assessed in male cancer survivors, who underwent sperm cryopreservation at the Department of Gynecological Endocrinology and Reproductive Medicine, Medical University Innsbruck, Austria from 01/2010 to 12/2018. Sexual functioning was assessed between 03-12/2020 via two questionnaires: Aging Male Score (AMS) and International Index of Erectile Function (IEEF-EF).
Participants/materials, setting, methods: Thirty-five cancer survivors (testicular cancer: n=16 [45.7%], hematological malignancies: n=15 [42.9%], others: n=4 [n=11.4%]) filled in two questionnaires (AMS and IEEF-EF) during routine follow-up visit at the Department of Gynecological Endocrinology and Reproductive Medicine, Medical University Innsbruck and the Department of Urology, Medical University Innsbruck, Austria. Moreover, sperm quality was assessed and normozoospermia was defined in accordance with the 2010 WHO criteria (sperm concentration ≥15 million/mL, progressive motility ≥32%, and ≥4% normal morphology).
Main results and the role of chance: Mean age at sperm cryopreservation and follow-up visit was 25.1±4.2 and 31.9±6.3 years, respectively with a mean follow-up time of 81.4±12.5 months. Rate of erectile dysfunction was low (75.0% no dysfunction, 15.6% low dysfunction, 3.1% low-moderate dysfunction, 3.1% moderate, 3.1% severe dysfunction). Moreover, AMS score indicated no, low, moderate and severe symptoms consistent with a low testosterone level in 77.1%, 8.6%, 2.9%, and 2.9% of patients, respectively. Oligozoospermia was observed in up to 48% of the patients with TM and in only 23% patients with HM. Patients with TM showed significantly reduced sperm count (18.7 × 106/mL [5.3–43.0]) and total sperm count (42.4 × 106/ejaculate [13.3–108.5]) compared to HM (p=0.03). There was no difference in sexual functioning between patients with HM or TM. Sexual functioning did no correlate with sperm count, progressive motility or morphology.
Limitations, reasons for caution: Although the study may be limited by its small sample size, it is the first to assess a correlation of sperm quality and sexual dysfunction in cancer survivors.
Wider implications of the findings: As every fourth male cancer patient suffers from impaired sexual functioning after gonadotoxic treatment, this important topic should be addressed in clinical and scientific future. Future studies should focus on both, somatic and psychosomatic reasons for sexual dysfunction.
Trial registration number: none

P-089 Paternal age affects the performance of EAU guidelines for genetic testing in infertile men: implications for candidate selection

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Study question: We aimed at challenge EAU Guidelines for genetic testing performance in infertile men according to normal vs. advanced paternal age (APA).
Summary answer: EAU Guidelines perform better in karyotype analysis (KA) and Y-chromosome microdeletions (YCM) investigation in men above 35 years of age.
What is known already: EAU Guidelines for genetic testing in infertile men recommend specific threshold for candidate selection for both KA (<10 million spermatozoa/ml) and YCM (<5 million spermatozoa/ml). However, paternal age is not taken into account for candidate selection in this setting.
Study design, size, duration: Data from 2188 infertile men (according to WHO definition) consecutively evaluated at a single academic centre were analysed.
Participants/materials, setting, methods: Demographic, clinical and laboratory data were analysed. Semen analyses were based on 2010 WHO criteria. Advanced age was defined as above 35. All men underwent KA and YCM testing. EAU Guidelines were validated in our cohort and according to APA. Specificity, sensitivity, and AUC were estimated for all scenarios. A Wald-type test compared AUC according to APA for KA and YCM. Decision curve analysis (DCA) estimated the benefit of using EAU Guidelines according to APA.
Main results and the role of chance: Median (IQR) paternal age was 37 (34-41) years. Advanced paternal age was found in 1306 (60%) of included men. Prevalence of KA and YCM was 4% (48 men) and 1% (13 men), respectively. EAU Guidelines sensitivity, specificity and AUC in the overall population were 85%, 47% and 66% for KA, whereas they were 100%, 57% and 80% for YCM. When stratifying according to APA, EAU Guidelines performed better in men over 35 both in terms of KA and YCM detection. Specifically, AUC for KA detection in men with APA was 70% vs. 63% in younger men (p=0.04). AUC for YCM detection in men with APA was 82% vs. 79% in younger men (p=0.03). DCA confirmed higher net benefit in using EAU Guidelines in old vs. young men for the detection of both KA and YCM.
Limitations, reasons for caution: It is a retrospective analysis at a single, tertiary-referral academic centre, thus raising the possibility of selection biases.
Wider implications of the findings: EAU Guidelines for genetic testing in infertile men perform differentially according to APA. KA and YCM are better detected in older men, likely due to a wider pool of confounding etiological factors in young men. These results suggest the implementation of more accurate predictive models in younger men.
Trial registration number: Not Applicable

P-090 Cumulative live birth rates (CLBR) per embryo transfer, embryo transferred and inseminated oocyte are not affected by paternal age in infertile male donor egg cycles

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Study question: Is reproductive success measured as CLBR per inseminated oocyte, per embryo transfer and per embryo transferred affected by paternal age in donor egg IVF-ICSI cycles?
Summary answer: Paternal age does not significantly affect reproductive outcomes measured by CLBR per inseminated oocyte, embryo transfer and embryo transferred in donor egg IVF-ICSI cyles.
What is known already: In recent years, the delay in the start of formation of a family has led to an increase of the average male age at which the first child is conceived. Therefore, there is a growing interest on the study of the impact of male age on the reproductive outcomes in assisted reproduction cycles (ART). Several studies have evaluated the effect of paternal age on reproductive outcomes using donor egg cycles to control for female factors. However, the results obtained on this topic are still controversial leading to a need of more research about it, which this study tries to address.
Study design, size, duration: This retrospective observational multicentric cohort study has included donor IVF-ICSI treatments (n=1539) performed to couples with etiology of male infertility (non-normozoospermic) in Spain IVIRMA clinics between January 2008 and March 2020 using patients' own sperm sample. Paternal age ranged from 28 to 74 years. The study population was categorized in 5 groups following the criterion of homogenizing the number of observations between groups 28-38 (A), 38-41 (B), 41-44 (C), 44-48 (D) y 48-74 (E) years.
Participants/materials, setting, methods: Considering that male age could be a factor affecting reproductive outcomes, we evaluated men with different age that performed a donor IVF-ICSI treatment with their own semen, etiology of male infertility and known age. Data was exported in order to obtain the clinical database and Kaplan-Meier was used for data analysis. P<0.05 was considered statistically significant. We measured reproductive success by CLBR per embryo transfer, per embryo transferred and per inseminated oocytes until live birth.
Main results and the role of chance: This study considered approximately 836 patients and 1411 embryo transfers. The CLBR per inseminated oocyte showed no significant difference between the study groups: A (3.09%, 42.61%, 71.96%), B (4.53%, 39.76%, 84.19%), C (5.89%, 47.04%, 78.61%), D (2.99%, 46.7%, 73.15%) and E (3.89%, 38.39%, 78.85%) for 7, 12 and 17 inseminated oocytes, respectively. In terms of CLBR per embryo transfer, the results obtained for each of the age groups were: A (51.55%, 70.69%, 92.18%), B (50.40%, 78.13%, 100.00%), C (53.68%, 71.69%, 100.00%), D (51.71%, 79.72%, 100.00%) and E (46.69%, 60.02%, 70.01%) for 3, 5 and 7 embryo transfers, respectively. No statistically significant differences were found among the studied age groups. CLBR per embryo transferred also did not show statistically significant differences between the age groups: A (8.43%, 53.34%, 72.13%), B (8.55%, 44,67%, 71.65%), C (10.40%, 53.94%, 72.49%), D (7.25%, 43.61%, 75.12%) and E (8.21%, 45.99%, 64.55%) for 1, 4 and 7 embryos transferred, respectively. Therefore, no significant differences were found in the number of inseminated oocytes, embryo transfers and embryos transferred needed to achieve a live birth between the age groups (p>0.05), suggesting that maybe paternal age has no relevant clinical effect on donor egg cycles with our categorization.
Limitations, reasons for caution: The retrospective nature of this study leads to biases derived from the clinical practice and to the presence of missing data (limiting sample size). Moreover, this study included donor egg cycles for controlling female factors, so this limits the generalization of our results to a population of young women.
Wider implications of the findings: Our study showed that the reproductive success measured as CLBR per embryo transfer, embryo transferred and inseminated oocytes was not statistically significant different among the studied age groups in donor egg cycles. Therefore, considering our study setting, paternal age does not affect reproductive success, however further studies should be done.
Trial registration number: NA

P-091 Magnetic Activated Cell Sorting (MACS) improves euploid blastocysts rate in pre-implantation genetic testing cycles with high levels of sperm DNA fragmentation and advanced paternal age.

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Study question: Can MACS increase euploid blastocyst rate in Pre-implantation Genetic Testing (PGT) cycles for AMA-APA (Advanced Maternal-Paternal Age) in patients with high sperm DNA fragmentation (SDF)?
Summary answer: A slight increase in euploid blastocyst rate was found using MACS in infertile patients with high SDF undergoing PGT cycles compared to the control group.
What is known already: Many authors have shown a close correlation between the presence of apoptotic markers on spermatozoa and the failure of assisted reproduction treatments. In normal physiological conditions, apoptotic spermatozoa with phosphatidylserine (PS) residues externalized on the plasma membrane, are eliminated along female genital tract, preventing oocyte fertilization. MACS eliminates apoptotic sperm whit PS residues using superparamagnetic microbeads conjugated with annexin V. This technique reduces the proportion of sperm with high rates of SDF and can be used to maximize ART

procedures results. MACS application improves sperm quality, fertilization, cleavage and pregnancy rates reducing miscarriage rate.
Study design, size, duration: From June to November 2020, 10 couples in which MACS was applied to select non-apoptotic spermatozoa, were randomly enrolled in our study (MACS group) and 8 couples without MACS were considered as controls (No-MACS Group). All couples in both groups underwent a PGT cycle and had high sperm DNA Fragmentation (> 20%). A higher rate of euploid and diploid-euploid mosaic blastocysts were obtained in the MACS group compared to the control group.
Participants/materials, setting, methods: Patients with severe oligoasteno teratozoospermia were excluded. MACS protocol was performed as follows: semen sample was analyzed (WHO 2010) and washed with buffered medium; pellet was removed and a swim-up was performed. Retrieved spermatozoa were washed with a binding buffer (Miltenyi Biotec), centrifuged (400 g x 4 minutes) and supernatant discarded. Pellet was covered with Annexin-V and re-suspended. After 15 minutes incubation at room temperature, the sample was eluted through the column and collected for ICSI.
Main results and the role of chance: In MACS group, female and male mean age ± SD were 41.6 ± 2.1 and 43.5 ± 7.3, respectively. Female and male mean age ± SD were 41.7 ± 2.8 and 44.6 ± 8.1 in the No-MACS group, respectively. In MACS and No-MACS groups, injected oocytes were 44 and 35, fertilized oocytes were 32 (72.3%) and 27 (77.1%) (NS), blastocyst formation rates were 71.8% (23/32) and 48.1% (13/27) (NS), respectively. In No-MACS group, only 1 euploid and 1 diploid-euploid mosaic blastocysts were obtained (1/13 = 8%) (NS). In MACS group, 4 euploid blastocysts were formed (4/23 = 17.4%) whereas mosaic diploid-euploid blastocysts were 3/23 (13.0%) (NS). Aneuploid blastocysts were 16/23 (69.6%) in MACS group and 11/13 (84.6%) in No-MACS group (NS).
Limitations, reasons for caution: AMA and APA of couples enrolled should be considered as a limit of the study. A larger number of patients and biopsied blastocysts are needed to analyze clinical results and perform a robust statistical analysis establishing if MACS is useful to improve transferable blastocyst rate in patients with high SDF.
Wider implications of the findings: MACS is useful to select non apoptotic sperms; although fertilization, cleavage and blastocyst rates are not improved, aneuploid blastocysts rate slightly decreases using MACS. It l possible that, selecting spermatozoa free from PS residues, MACS allows to choose spermatozoa with a better DNA packaging, thus affecting the embryo ploidy.
Trial registration number: non applicable

P-092 The more, the merrier: does ejaculatory frequency influence seminal parameters in oligospermic men?

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Study question: Does ejaculatory frequency during the three months preceding semen collection influence semen parameters in oligospermic men?
Summary answer: A frequency of 2-3 ejaculations/week during the three months preceding semen collection significantly optimizes sperm motility, without any reduction in sperm concentration.
What is known already: Male gametes undergo crucial physiological and biochemical changes during epididymal transit, but a longer storage is known to have negative effects on semen quality, especially on motility. Previous studies focused on abstinence prior to semen collection, while few data are available on the effect of ejaculation frequency. On one hand, a longer storage could increase exposure to reactive oxygen species and a pro-inflammatory environment, with a reduction in vitality and motility. On the other, an increased ejaculation frequency could cause a reduction in sperm volume and concentration. The effects of ejaculatory frequency are particularly understudied in men with oligospermia.
Study design, size, duration: This is a retrospective study performed at a tertiary level public infertility center. We included all semen samples, collected both for diagnostic purposes and ART cycles between September 2019 and September 2020, with a sperm concentration of 15 million/ml or less, and an abstinence of 3- 5 days. Exclusion criteria were surgically collected or collected for fertility preservation semen samples.
Participants/materials, setting, methods: Standard demographic and clinical data were recorded, as well as semen parameters. Ejaculation frequency was considered "optimal" (at least 2-3/week) or "reduced" (<1/week). The potential predictive role of ejaculation frequency, age, BMI, smoking habits, previous cryptorchidism, varicocele, days of abstinence on semen parameters was evaluated by univariate and then by multivariate analysis for all factors significant in the univariate models. P< 0.05 was considered statistically significant. Main results and the role of chance: Out of 738 men, 491 reported an optimal ejaculation frequency, 247 had <1 ejaculation/week, no one reported everyday ejaculations. Total sperm mobility (35.91±22.84% vs. 32.28±16.91%, p=0.02) and sperm rapid progressive motility (5.56±6.09% vs. 4.20±6.1%, p=0.006) were significantly higher in the group with optimal ejaculation frequency. Ejaculation frequency remained predictive of total mobility (p=0.04) and rapid progressive mobility (p=0.03) in a multivariate linear regression model with age and sperm concentration. Sperm volume (2.92±1.56 ml vs. 2.91±1.54 ml, p=NS) and concentration (5.74±5.05 mil/ml vs. 6.05±4.78 mil/ml, p=NS) did not significantly differ depending on the declared ejaculation frequency.
Limitations, reasons for caution: The study is retrospective and ejaculatory frequency was self-reported as an estimate of the mean of the number of ejaculations per week.
Wider implications of the findings: Optimizing ejaculatory frequency may improve ART outcomes as well as success of spontaneous conceptions. There is no reason to limit ejaculatory frequency in oligospermic men for a hypothesized benefic in sperm concentration.
Trial registration number: not applicable

P-093 The use of donor sperm improves post-ICSI live birth rates in advanced maternal age women

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Study question: Can the use of donor sperm improve post-ICSI live birth rate in advanced maternal age (AMA) patients?
Summary answer: The use of donor sperm increases post-ICSI live birth rate while substantially reducing abortion occurrence in AMA patients.
What is known already: Oocyte DNA repair capacity decreases with maternal age, when sperm DNA integrity is particularly important to avoid the transfer of gene truncations and *de novo* mutations to the zygote. Optimal DNA repair activity in the zygote requires paternal inheritance of 8-oxoguanine DNA glycosylase (OGG1), a rate-limiting enzyme in the base excision repair pathway. However, the involvement of paternal aging and sperm quality in the severe drop in fertility observed in AMA patients has not been addressed. While strategies to mitigate the impact of AMA on fertility have exclusively targeted oocyte quality, the sperm contribution in this scenario remains somehow neglected.
Study design, size, duration: Retrospective, multicentric, international study including 755 first ICSI cycles with patients' own oocytes achieving a fresh ET between 2015 and 2019, 337 of which using normozoospermic partner semen and 418 using donor sperm. The association of sperm origin (partner *vs.* donor) with live birth was assessed by univariate/multivariate analysis in non-AMA (<37 years, n=278) and AMA (≥37 years, n=477) patients. ICSI outcomes were compared between partner and donor sperm in non-AMA and AMA patients.
Participants/materials, setting, methods: The study was conducted in 3 fertility clinics including 755 Caucasian patients aged 24 to 42 years. Univariate/multivariate analyses were performed to test the association of sperm origin with live birth; infertility factor, maternal age, oocyte yield and number of embryos transferred were included in the model as confounding variables. In addition, ICSI outcomes were compared between donor and partner sperm groups with the Chi-square (percentages) or with the Wilcoxon sum rank (continuous variables) tests.

Main results and the role of chance: The multivariate analysis revealed that the use of donor sperm was positively and independently associated with live birth occurrence in AMA [1.82 OR (1.08-3.07) 95% IC; p=0.024], but not in non-AMA patients [1.53 (0.94-2.51); p=0.090]. Maternal age [0.75 (0.64-0.87); p<0.001], number of MII oocytes recovered [1.14 (1.05-1.23); p=0.001] and number of embryos transferred [1.90 (1.27-2.86); p=0.002] were also independently associated with live birth in AMA patients. Live birth and delivery rates were 70-75% higher, while miscarriage rate was less than half in donor sperm compared to partner sperm AMA cycles (LBR: 25.4% *vs.* 14.5%, p=0.003; DR: 22.5% *vs.* 13.5%, p=0.008; MR: 18.0% *vs.* 39.5%; p=0.009). Implantation (17.4% *vs.* 13.5%; p=0.075) and clinical pregnancy rates (27.5% *vs.* 22.3%; p=0.121) did not significantly differ between sperm donation and partner sperm AMA cycles. Male age was substantially lower (23.6 ± 5.2 *vs.* 41.4 ± 5.0; p<0.0001) and oocyte yield was higher (5.1 ± 3.1 *vs.* 4.3 ± 2.6; p<0.0001) in sperm donation compared to partner sperm AMA cycles, while maternal age did not vary (39.8 ± 1.6 *vs.* 39.6 ± 1.7; p=0.348).

Limitations, reasons for caution: This study is limited by its retrospective nature and by differences in patients' profiles between sperm donation and homologous cycles, although this variation has been controlled for in the statistical analysis.

Wider implications of the findings: The findings suggest that donor sperm can improve live birth rates by drastically reducing miscarriage occurrence in AMA patients. Therefore, the present results may influence AMA treatment decisions and, above all, contribute for AMA patients to achieve a healthy birth.

Trial registration number: not applicable

P-094 Kidney function impairment in primary infertile men

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Study question: We investigated the prevalence of kidney function impairment in a homogeneous cohort of white-European primary infertile men.

Summary answer: Mild kidney function impairment characteristics were found in 9% of asymptomatic and unaware patients presenting for primary infertility investigation.

What is known already: Infertile men have shown a worse overall health status compared to the fertile counterpart. We investigated the prevalence of kidney function impairment in a homogeneous cohort of white-European primary infertile men.

Study design, size, duration: In this corss-sectional study, complete clinical and laboratories data from a cohort of 557 consecutive men aged >18 years, presenting for primary infertility investigation were analyzed.

Participants/materials, setting, methods: Comorbidities (as scored with the Charlson Comorbidity Index (CCI)) were collected in each patient. Primary outcome was the presence of functional impairment of the kidney (defined as an estimated glomerular filtration rates <90 ml/min/1.73m², according to the Kidney Outcomes Quality Initiative). The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) function was used for eGFR calculation. Logistic regression analyses tested the association between kidney function impairment and clinical and semen features.

Main results and the role of chance: Of 557, 51 (9.3%) patients depicted at least a mild loss of kidney function (eGFR<90 ml/min /1.73m²). Median [interquartile (IQR)] age was 38 (35-42) years for normal kidney function group vs. 41 (36-46.5) years for those with impaired renal function (p<0.001). Those with impaired renal function had also a higher BMI [26.1 (24.4-27.8) vs. 24.9 (23.2-26.8); p=0.002] and higher numbers of comorbidities [CCI≥ 1 in 11 (21.5%) vs. 40 (7.9%) patients (p<0.001)]. Of note, they had more frequently history of hypertension [10 (19.6%) vs. 31 (6.1%), p=0.001]. Groups did not differ in terms of hormonal and semen features. At logistic regression analysis, older age and CCI≥1 were associated with a higher risk of impaired eGFR (OR 1.06; 95%CI 1.01-1.11; p=0.016 and OR 2.41; 95%CI 1.06-5.15; p=0.028, respectively)(table). No association was found between sperm parameters and eGFR impairment (all p>0.05), after accounting for age, CCI, BMI, FSH, testicular volume, and varicocele**.**

Limitations, reasons for caution: Mild kidney function impairment characteristics were found in 9% of asymptomatic and unaware patients presenting for primary infertility investigation. Age and the rate of comorbidities are associated with reduced eGFR. Wider implications of the findings: This novel finding confirms growing data on a significant association of male infertility with a poorer overall male health status.

Trial registration number: not applicable

P-095 Outcomes and predictive factors of successful salvage microdissection testicular sperm extraction (mTESE) after failed TESE in men with non-obstructive azoospermia: results from a multicenter study

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Study question: We assessed the outcome and predictors of successful salvage microdissection testicular sperm extraction (mTESE) in non-obstructive azoospermia (NOA) men previously submitted to unfruitful classic (cTESE).

Summary answer: The sperm retrieval rate at salvage mTESE was almost 50%. Hypospermatogenesis and low FSH values were associated with positive outcomes at salvage mTESE

What is known already: In men with NOA testicular sperm can be retrieved using cTESE in approximately 50% of cases. mTESE has been proposed as a salvage treatment option for men with a previously failed TESE, but data are scarce.

Study design, size, duration: Multicenter, cross-sectional study. Complete data from 61 NOA men who underwent mTESE after a failed cTESE between 01/2014 and 10/2020, at 6 tertiary referral centers in Italy were analysed.

Participants/materials, setting, methods: All men underwent testicular ultrasound, hormonal and genetic blood testing. Histopathological diagnosis from TESE was collected in every man. Semen analyses were based on the 2010 WHO reference criteria. mTESE was performed according to the technique of Schlegel et al. (1999). Descriptive statistics and logistic regression models were used to investigate potential predictors of positive sperm retrieval (SR+) after salvage mTESE.

Main results and the role of chance: Overall, median (IQR) age and testicular volume were 35 (31-38) years and 10 (6-15) ml, respectively. Baseline serum FSH and total testosterone levels were 17.1 (8.6-30.4) mUI/mL and 4.7 (3.5-6.4) ng/mL, respectively. Sertoli-cell-only (SCO) syndrome, maturation arrest (MA) and hypospermatogenesis were found in 24 (39.3%), 21 (34.4%) and 16 (26.2%) men after cTESE, respectively. Spermatozoa were retrieved in 30 (49.2%) men at salvage mTESE. Patients with a diagnosis of hypospermatogenesis had a higher rate of SR+ [12/16 (75%)] than those with MA [12/21 (57.1%)] and SCOS [6/24 (25%)] after salvage mTESE (p<0.01), which was bilateral in 36 (59%) cases. FSH

was higher [16.5 (8-22) vs. 8.9 (5-13) mUI/mL, p<0.01] in SR- patients compared to SR+. No difference in clinical characteristics was found between patients with SR+ and SR- at salvage mTESE. There were no significant complications after mTESE. Multivariable logistic regression analysis showed that hypospermatogenesis (OR 9.7; p<0.01) and low FSH levels (OR 0.9, p<0.001) were independent predictors of SR+ after salvage mTESE, after accounting for age.
Limitations, reasons for caution: Despite we analysed one of the largest series of salvage mTESE, the samples size is too small to draw general conclusions. Because of the multicenter nature of the study we cannot rely on standardization of surgical techniques for TESE.
Wider implications of the findings: This is one of the larger studies on salvage mTESE. The selection of patients for salvage mTESE is of critical importance.
Trial registration number: na

P-096 Real-time ranking of single spermatozoa using artificial vision analysis of complex motility patterns during ICSI aimed at improving fertilization and blastocyst development

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Study question: Can real-time artificial vision identify beneficial movement patterns of single spermatozoa in a cohort visualized in PVP during ICSI possibly enhancing fertilization and embryo development?
Summary answer: Artificial vision seems able to identify advantageous movement patterns of individual spermatozoa having a significant impact on both normal fertilization and blastocyst formation.
What is known already: Spermatozoa isolated from poor semen may reduce the quality of embryo development and blastocyst formation. Normal motility is dependent on general sperm morphology and characteristic movement of the flagellum enabling forward mobility. Spermatozoa roll as they swim. It is known that this rotational motion around their longitudinal axis promotes rheotaxis, which is a mechanism that allows the sperm to navigate to the site of fertilization. Therefore, it is possible that the characteristics of the rotational movement are related to sperm quality.
Study design, size, duration: Non-intervention study based on a cohort of 132 videos of in-vitro fertilization treatments with ICSI during which the sperm selection process was recorded up to sperm injection. The study was performed at one IVF center within a 6-month period. Injected spermatozoa and their corresponding oocytes were individually assessed from fertilization to blastocyst formation. Videos, where spermatozoa selected for injection could not be identified, were excluded. Relevant outcomes included normal fertilization (2PN), and blastocyst formation.
Participants/materials, setting, methods: Using a digitizer attached to an optical microscope (640 x 480 pixels), videos were recorded to include the sperm selection process, immobilization, and subsequent injection following standard ICSI protocols. Individual spermatozoa motility features were extracted using a proprietary computer-vision algorithm (SID, IVF 2.0 LTD). The rotational movements of spermatozoa were inferred by computing the variations of the mean intensity of the sperm in the video-sequence across time (MI).
Main results and the role of chance: Based on SID's analysis, we found statistically significant differences between the median prominences of the MI of those injected spermatozoa that resulted in successful fertilization in comparison to those with failed fertilization (p-value=0.029, 28 negative fertilization, and 71 positive fertilization) using a one-tailed t-Student test with a significance level of 5%. We also found statistically significant differences between the median prominences of the MI of those spermatozoa that resulted in blastocysts in comparison with the spermatozoa-oocyte cohorts which didn't reach the blastocyst stage (p-value 0.004, 51 with negative blastocyst formation and 48 with blastocyst formation).
Limitations, reasons for caution: The size of this database is modest, therefore a larger study with multiple clinics will be necessary to confirm the findings. Large prominence does not necessarily assurance successful fertilization or blastocyst formation since there may be other factors such as oocyte quality or the ICSI technique.
Wider implications of the findings: Objective assessment of sperm rotational movement is difficult to quantify and to be objectively assessed during standard sperm selection. Real-time artificial vision tools such as SID could assist embryologists during the sperm selection process for ICSI.
Trial registration number: NA

P-097 The impact of SARS-CoV-2 on male gonadal function. A longitudinal study

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Study question: Can severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) be detected in the semen of SARS-CoV-2 positive men, and does SARS-CoV-2 infection affect male reproductive function?
Summary answer: No SARS-CoV-2 RNA was detected in semen. An impact of SARS-CoV-2 infection on semen quality and reproductive hormone profile awaits evaluation at 3+6 months follow-up.
What is known already: SARS-CoV-2 may use angiotensin-converting enzyme (ACE)2 as an entry point into the cell. As ACE2 is expressed in testicular tissue, it has been speculated that SARS-CoV-2 may affect the male reproductive system. A cohort study including 38 male COVID-19 patients showed that SARS-CoV-2 was present in the semen of six patients (15.8%) [Li *et al*., 2020]. Later studies including a total of 223 patients have not provided evidence of transmission of SARS-CoV-2 via semen. There are to date no available longitudinal studies on semen quality following SARS-CoV-2 infection.
Study design, size, duration: Longitudinal cohort study including 50 non-hospitalized men from the general population in the Capital Region of Denmark. All participants had a confirmed SARS-CoV-2 infection by reverse-transcription polymerase chain reaction (RT-PCR) on oropharyngeal swab material within the last week. The presence of SARS-CoV-2 in semen samples by RT-PCR, semen parameters and reproductive hormone profile were assessed at inclusion and at 3 + 6 months follow-up. SARS-CoV-2 antibody levels were assessed 3-5 weeks after inclusion.
Participants/materials, setting, methods: SARS-CoV-2-positive males (age 18-60 years) were included. Oropharyngeal and semen samples were tested by RT-PCR applying the E-Sarbeco primers and probe published by Corman *et al.* 2020 and adapted to TaqMan Fast Virus 1-step master mix and LightCycler 480 as previously reported by Jørgensen *et al.* 2020. SARS-CoV-2 antibodies were detected using the serological immunoassay from Shenzhen YHLO Biotech on the iFlash 1800. Semen quality parameters were analysed according to World Health Organisation (WHO) standards.
Main results and the role of chance: To date, 25 men with a mean age of 35 years have been included in the study. SARS-CoV-2 RNA could not be detected in the semen samples of any of the 25 men at the time of inclusion. Twenty-one of the 25 men (84,0%) had a same day RT-PCR-confirmed SARS-CoV-2 infection in an oropharyngeal swab. RT-PCR cycle threshold (ct) values were distributed as follows: four (19,0%) were strongly positive (ct <25), 16 (76,2%) intermediately positive (ct 25-35) and one (4,8%) weakly positive (ct 35-45). The four men without PCR-confirmed SARS-CoV-2 infection all had a positive IgG response to SARS-CoV-2 at the time of inclusion. Longitudinal semen and reproductive hormone profiles analyses will be performed. Further studies are needed to prove whether SARS-CoV-2 can be transmitted to the male reproductive tract and whether SARS-CoV-2 infection may cause alterations of spermatogenesis and endocrine function.

Limitations, reasons for caution: Strengths of this study are the unselected population of men examined within a week after confirmed SARS-CoV-2 infection and the follow-up of semen parameters and endocrine profile. Limitations are the limited sample size and the fact that semen quality was not known before the participants were diagnosed with COVID-19.
Wider implications of the findings: Knowledge of viral detection and semen persistence of SARS-CoV-2 is essential for clinical practice and public health. There is a need for evidence-based counselling on the impact of SARS-CoV-2 infection for patients undergoing assisted reproduction technology and patients who have a need for semen cryopreservation.
Trial registration number: H-20027362

P-098 Use of Dimethylxanthine Theophylline in surgical retrieved sperms that do not recover motility after thawing

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Study question: Can the use of Theophylline recover motility of frozen surgically retrieved sperms in case of absence of motility after thawing?
Summary answer: Theophylline allows to recover motility of thawed surgically retrieved sperms. The utilization of sperms with or without pharmacological activation gives comparable clinical outcomes.
What is known already: Testicular sperm motility is usually poor. A method is needed to detect viable sperm for ICSI when motility is totally absent after freezing/thawing. Hypo-osmotic swelling test, mechanical touch technique, laser-assisted immotile sperm selection, birefringence-polarization microscopy and exposure to pharmacological stimulation are techniques used for this purpose. Among pharmacological agents Dimethylxanthine Theophylline is a phosphodiesterase inibitor that improves sperm motility by promoting an increase in intracellular cyclic AMP levels. Few studies report that it is efficient for recovery of sperm motility in cases of thawed testicular and retrograde ejaculation samples improving reproductive outcomes.
Study design, size, duration: Retrospective analysis of sixty frozen surgical sperm cycles (45 patients) utilized from February 2018 to November 2020. After thawing, samples were divided in two Groups according to motility recovery. Group A: presence of motility, Group B: absence of motility. Group B was treated with Theophilline and motility was re-assessed after incubation. Activated sperms were utilized for ICSI when available. Sperm motility recovery, fertilization, pregnancy rate/transfer, implantation and miscarriage rate were evaluated in both Groups.
Participants/materials, setting, methods: Surgical specimens were treated and concentrated in SpermRinse™ Medium (Vitrolife) and then cryopreservated in nitrogen vapor in TEST Yolk Buffer (Irvine Scientific). After thawing, only samples with no motility recovery were treated with a brief incubation in Theophylline (GM501 SpermMobil, Gynemed) and washed in Polyvinylpyrrolidone (ICSI™ Vitrolife) before injection. ICSI was performed in all cases approximately 4-5 hours after sperm thawing. After fertilization check, transfer was scheduled in day 2.
Main results and the role of chance: Women's age Group A (34,39±2,29 M±SD) and group B (35,87±4,34 M±SD) and men's age Group A (37,31±5,12 M±SD) and group B (40,89±8.15 M±SD) were not significantly different (*P*=.328 and *P*=.218) respectively.

Group A: 13/60 cycles (21.7%) (9 patients). Pre freezing and post thawing total motility percentage were 34.0±19.0 (M±SD) and 13.5±15.6 (M±SD) respectively (39.8% recovery). Group B: 47/60 cycles (78.3%) (36 patients). Pre freezing total motility percentage was 5.3±8.5 (M±SD) and no motility was recovered post thawing (0%). After treatment with Theophylline total motility was 1.8±1.8 (M±SD) (33.5% recovery). Motile sperms were utilized in all cases except from two in the Group B.

Number of injected oocytes was 2.8±1.1 (M±SD) in Group A and 4.3±3.1 (M±SD) in Group B (*P*=.004) respectively.

Fertilisation rate (63.1% and 45.4%, *P*=.066), Number of embryos transferred (1.8±0.7 M±SD and 1.6±0.7 M±SD, *P*=.271), Pregnancy rate/Transfer (54.5% and 37.1%, *P*=.502), Implantation rate (30.0% and 27.8%, *P*=.919) and Miscarriage rate (33.3% and 30.7%, *P*=.675) were not statistically significant between Group A and B respectively.

In the two cases of group B injected with immotile sperm, fertilization rate was 0% (0/3) and 50% (2/4).
Limitations, reasons for caution: A larger study is needed to investigate the recovery of sperms motility (and/or their activation) and clinical outcomes, in particular referring to the origin of sampling (epididymal aspirate and testicular tissue) and type of azoospermia (obstructive and non-obstructive).
Wider implications of the findings: Theophylline is an effective tool for sperm motility recovery after thawing allowing to inject viable sperm and facilitating laboratory handling.
Trial registration number: not applicable

P-099 MYD88 dependent pathway through TLR 1,2 and 6 activation play a role in interaction of high DNA fragmented human sperm with fallopian tube epithelial cells

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Study question: How does sperm with high DFI disrupt the female reproductive system?
Summary answer: Sperm with damaged DNA could disrupt the female reproductive system by production of high inflammatory factors through activation of TLRs and MyD88-dependent pathway .
What is known already: Previous studies have also shown that the interaction between sperm and fallopian tube is a complex process and activates a number of immune mechanisms. Many TLRs, including TLR1, TLR2, and TLR6 are expressed in fallopian tube epithelial cells. TLRs have an important role in the immune interaction between fallopian tube and sperm. They are also involved in sperm capacitation, fertilization and pregnancy. Examining this interaction may lead us to valuable data.
Study design, size, duration: In this study, 10 Recurrent Implantation failure couples with high DFI (>30% by SCSA evaluation) and 10 healthy donors men with low DFI (<30%) were considered as high DFI group and low DFI group respectively. After fresh semen preparation, sperm were co-cultured with human fallopian tube epithelial cell line(OE-E6/E7) for 24 hours**.**
Participants/materials, setting, methods: RNA were extracted from the cell line and PCR array for human innate and adaptive immune responses was performed by RT2 profiler PCR array.
Main results and the role of chance: Analysis of PCR array data showed that the expression of TLR-1, TLR-2, TLR-6, MYD88, TIRAP, IRAKS,TRAF6, MAPKS, NF-KB, G-CSF, GM-CSF, CXCL8, CXCL10, CCL2, IL-6, IL-1, TNFa in high DFI group was significantly higher than control group. All these factors are involved in the MyD88-dependent pathway. Our research suggests that the MyD88-dependent pathway through TLR1,2 and 6 activation may be the main inflammatory pathway activated by sperm with high DFI. The released DAMPs through damaged sperms were recognized by TLRs on the epithelial cells. Following the TLR1,2 and 6 signaling, and MYD88 activation, epithelial cells were producing the inflammatory cytokines which result in the neutrophils infiltration. In the lumen of the fallopian tube, the neutrophils were activated via TLR1/TLR2, complexes that lead to the neutrophils activation, phagocytosis, NET formation, and apoptosis.
Limitations, reasons for caution: Studying with a larger sample size and examining the final factors at the protein level will be better to detect the effect of sperm DNA fragmentation on fallopian tube.
Wider implications of the findings: MyD88-dependent pathway could be one of the mechanisms that involved in interaction of high DNA fragmented sperm with female reproductive tract.
Trial registration number: not applicable

P-100 Metabolic, hormonal, and inflammatory status in sub-fertile men with obesity and diabetes

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Study question: To evaluate the association between sperm functionality parameters and biochemical, hormonal, and inflammatory indices in obese and diabetic men.
Summary answer: Metabolic changes,hormonal dysfunction,and the presence of inflammatory mediators might be considered possible mechanisms in the development of sub-fertility in obese and diabetic sub-fertile men
What is known already: Although the higher prevalence of subfertility in obese and diabetic men during the reproductive age is evident, the mechanisms by which obesity and diabetes mellitus (DM) cause male infertility are not entirely understood. Several pathways might be involved in the role of obesity in semen quality, thereby inducing alterations in hormonal profiles, abnormal lipid metabolism, and possibly the formation of inflammatory cytokines, ultimately leading to impaired sperm function
Study design, size, duration: We enrolled normal weight (BMI<25 kg/m2) and non-type-2 diabetic (control=40), obese and non- type-2 diabetic (obese=40), non-obese and type-2 diabetic (Lean-DM=35), and obese and type-2 diabetic (Obese-DM=35) sub-fertile men, aged 20-50 years, referring to Royan infertility clinic (Tehran, Iran) from March to September 2014
Participants/materials, setting, methods: After enrollment and receiving informed consent, all men underwent face-to-face private interviews. The obesity-associated markers, insulin resistance, beta-cell function, hormonal and lipid profile, inflammatory indices, and semen analysis were assessed in four experimental groups. Semen analysis was examined after 2–5 days of sexual analysis). abstinence based on WHO-recommended methods by CASA system (computer-assisted sperm
Main results and the role of chance: Main results and the role of chance: Our finding showed that diabetic markers were significantly increased in two diabetic groups, while obesity indices were markedly increased in two obese groups. Conventional sperm parameters were significantly lower in obese DM, lean DM, and obese groups compared with the control (p<0.05). Serum levels of total testosterone (TT) and sex hormone-binding globulin (SHBG) were significantly lower in men with obesity and DM compared with the control (p< 0.05).There was a significant difference in the concentration of high-sensitivity C-reactive protein (hs-CRP) among four experimental groups.

Moreover, serum leptin was significantly increased in obese DM, lean DM, and obese groups. Serum insulin levels had a positive correlation with metabolic-associated indices (WC, BMI, FBS,HbA1c,and HOMA-IR), as well as hs-CRP levels, whereas it had a negative correlation with count, motility, and morphology. There is also a negative association between metabolic-associated indices (WC, BMI, FBS, HbA1c, and HOMA-IR) and semen parameters.
Limitations, reasons for caution: It was better to evaluate inflammatory biomarkers be examined in other tissues
Wider implications of the findings: The results of this study demonstrated the association of metabolic changes, hormonal dysfunction, and inflammatory responses with the semen parameters of sub-fertile men with obesity and diabete.
Trial registration number: not applicable

P-101 Comparative assessment of laparoscopic, microsurgical varicocelectomy, and antioxidant therapy alone in Infertile men with pathozoospermia

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Study question: Which treatment option is better in men with clinical varicocele and pathozoospermia: laparoscopic, microsurgical varicocelectomy, or antioxidant (nutrient) therapy alone?
Summary answer: Microsurgical varicocelectomy and laparoscopy are more effective in pathozoospermia treatment than observation and nutrient therapy alone. Simultaneously, there are no differences between these surgical methods.
What is known already: It is known that varicocele may cause testicular dysfunction and infertility due to increased oxidative stress and sperm DNA damage. In recent meta-analyses comparing surgery versus follow-up in men with clinical varicocele and pathozoospermia, semen quality was better in the surgery group. However, it is unclear why varicocelectomy leads to sperm quality improvement only in 60-70% of cases, and real fertility in 30-40% of couples. The microsurgical technique leads to fewer complications compared with others, but there are no powerful RCTs to compare various techniques' efficacy. Simultaneously, the use of antioxidants may give a similar increase in pregnancy rates.
Study design, size, duration: This retrospective case-control study recruited 218 men from infertile couples with clinical varicocele and pathozoospermia who underwent microsurgical, laparoscopic varicocelectomy and antioxidant therapy alone at clinics of Moscow from January 2010 to December 2019.
Participants/materials, setting, methods: Clinical, laboratory data of patients in the groups: A) the observation group (n = 33), B) the group treated with nutrients alone (n = 63), C) the group of patients after microsurgical varicocelectomy (n = 86), and D) the group of patients who underwent laparoscopy (n = 36), were obtained. The sperm was evaluated according to WHO-2010, DNA fragmentation by chromatin dispersion in an agarose gel. We calculated standardized effect (Es) to determine study power.
Main results and the role of chance: After 3 months, varicocelectomy led to an increase in sperm concentration and motility: the median of the total progressively motile sperm count (TPMSC) increase in the group A was +0.4 million; B - +1.9 million; C - +17.1 million (p <0.05); D - +21.2 million (p <0.05). A clinically significant increase in this indicator after varicocelectomy was found in 2/3 of cases: 65% (; p<0.05) and 67% (D; p<0.05) with 38% (A) and 42% (B). Varicocelectomy led to a decrease in sperm DNA fragmentation by an average of 5.5% (p<0.05) with an improvement in 59% of patients. Simultaneously, a 3-month therapy with nutrients similarly decreased DNA fragmentation: 5.5% (p <0.05), 66% of improvement cases. The differences in surgery efficacy between C and D were insignificant (p>0.05). The laparoscopic surgery demonstrated higher standardized effect (Es) than microsurgical operation (Es = 0.70 and 0.44, with 0.29 in the patients receiving nutrients and 0.22 in the patients of the control group).
Limitations, reasons for caution: The main limitations were: 1) different sample (group) sizes and 2) insufficient power of the performed study (Es<0.8), which does not allow us to exclude a type II error – unreasonable rejection of differences.
Wider implications of the findings: In selected patients with varicocele and pathozoospermia, antioxidant therapy can be used as a monotherapy or as adjuvant therapy.
Trial registration number: Not applicable

P-102 GM-CSF (granulocyte-macrophage colony-stimulating factor) as a sperm medium supplement improves sperm quality in Oligoastenoteratospermia (OAT) men by activating the PI3K/Akt pathway

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Study question: Can GM-CSF as a sperm medium supplement improve sperm quality in OAT men?
Summary answer: GM-CSF can be used to improve sperm quality in OAT men by activating the PI3K/Akt pathway.
What is known already: OAT patients have very low sperm parameters, including morphology, count, and motility, which can adversely affect the results of assisted reproduction technologies. It seems the development of sperm media is necessary to improve the sperm parameters of these patients. GM-CSF is a natural growth factor produced by the reproductive organs, culture media supplemented with GM-CSF is widely commercially available and enhances the embryo development and implantation rate. Studies show that this growth factor in the semen of infertile men is lower than that of fertile men. However, there is no study to assess the effect of GM-CSF on sperm quality.
Study design, size, duration: In the present study, Semen specimens were collected from 20 OAT patients who have male infertility factors, according to WHO criteria. After the swim-up washing procedure, each of the samples is divided into two groups; experiment, and control. In the experimental group, samples are incubated with a medium containing 2 ng/ml GM-CSF for one hour, yet, in the control group, the sperms are incubated without GM-CSF for the same time.
Participants/materials, setting, methods: The sperm motility was examined with phase-contrast microscopy, Eosin-nigrosin staining method was used to assess sperm viability. The expression of sperm glucose transporters (GLUT 1, 3) was determined using immunofluorescent staining, the ratio of pAkt to total Akt was assessed by the Western blotting method. Image J software was used to quantify results; the data was analyzed by SPSS software. P-value<0.05 was considered statistically significant.
Main results and the role of chance: As compared to the control group, supplementation with GM-CSF improved sperm progressive motility, enhanced GLUT 1 and 3, and p-AKT/AKT expression (P<0.05). There was no significant difference between the viability of the control and experimental groups.
Limitations, reasons for caution: This study needs further investigations, including Real-time PCR for the genes of this signaling pathway which is ongoing.
Wider implications of the findings: We showed for the first time that GM-CSF can improve sperm quality by influencing motility and energy metabolism in spermatozoa which can be affected by increasing the phosphorylation of AKT. This growth factor could be an appropriate supplement in sperm media for OAT patients.
Trial registration number: IRCT20200519047508N1

P-103 Novel sperm preparation techniques compared with conventional preparation method

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Study question: Which sperm preparation technique separate the best quality sperm?
Summary answer: Microfluidic method improved the sperm parameters and decreased sperm DNA damage.
What is known already: About 40% of infertility issues are due to male factor. One of the known causes of male infertility is associated with low sperm parameters and high level DNA fragmentation. Sperm preparation techniques in ICSI procedures is used in order to obtain the best-quality sperm**.**
Study design, size, duration: The present study was designed to compare Microfluidic, Zeta potential, Magnetic Activated Cell Sorting (MACS) and Swim-up methods for sperm preparation and the effect of these methods on semen parameters and sperm DNA integrity in infertile men (n = 25) with a mean age of 38.
Participants/materials, setting, methods: In this study, each sample was divided into 4 groups, one part for preparing by Microfluidic method, one of them for preparing by Swim -up method, the other one was prepared by MACS and the last one was prepared by zeta potential. Then sperm count, viability, motility and morphology were assessed according to WHO 2010. DNA damage were assessed by Sperm DNA Fragmentation assay and sperm chromatin packaging assessed by CMA3 staining test
Main results and the role of chance: Sperm parameters including viability, motility, and morphology in the Microfluidic method were significantly improved and sperm DNA damage were significantly lower than three other methods (P-value <0.05). The sperm parameters and sperm DNA damage after preparation by MACS and Zeta potential methods were not significantly different however in the Swim-up method sperm parameters were lower than three other methods (P-value <0.05).
Limitations, reasons for caution: The fertilization and pregnancy rate of the resulting embryos are not available.
Wider implications of the findings: Our results showed that Microfluidic can be an effective way to improve sperm quality of infertile male compared to conventional preparation methods. We also found instead of the MACS method, we can use the Zeta potential method according to their costs, for sperm preparation during ART cycle.
Trial registration number: *

P-104 Assessment of sperm motility according to WHO classification using convolutional neural networks

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Study question: How does convolutional neural network (CNN)-predicted sperm motility correlate with manual assessment according to the WHO guidelines.
Summary answer: CNN predicts sperm motility comparable to reference laboratories in the ESHRE-SIGA External Quality Assessment Programme for Semen Analysis.
What is known already: Manual sperm motility assessment according to WHO guidelines is regarded as the gold standard. To obtain reliable and reproducible results, comprehensive training is essential as well as running internal and external quality control. Prediction based on artificial intelligence can potentially transfer human-level performance into models that perform the task faster and can avoid human assessor variations. CNNs have been groundbreaking in image processing. To develop AI models with high predictive power, the data set used should be of high quality and sperm motility assessment based on WHO guidelines.
Study design, size, duration: Videos of 65 fresh semen samples obtained from the ESHRE-SIGA External Quality Assessment Programme for Semen Analysis (from the period 2006-2018) were used in the development of the model. One video was captured for each semen sample. Sperm motility data was obtained from manual assessment of the videos according to WHO criteria by reference laboratories in the programme. Rapid progressive motility was also included. Ten-fold cross-validation was used to compensate for the relatively small dataset.
Participants/materials, setting, methods: The mean values of the reference laboratories were used. Sparse optical flow of the sperm videos was generated from each second of each video and fed into a ResNet50 convolutional neural network. For training, Adam was used to optimize the weights and mean squared error (MSE) to measure loss. For baseline, ZeroR (pseudo regression) was performed. Results are reported as MAE. For correlation analysis, Pearson's r was used.
Main results and the role of chance: Predicting sperm motility based on the optical flow generated from the videos, achieved an average MAE of 0.05 across progressive (0.06), non-progressive (0.04) and immotile sperm (0.05). The ZeroR baseline was 0.09, indicating that the method is able to capture the movement of the spermatozoa and predict motility with low error. Pearson's correlation between manually and AI-predicted motility showed r of 0.88, p<0.001 for progressive, 0.59, p<0.001 for non-progressive and 0.89, p<0.001 for immotile sperm. When predicting rapid progressive motility, the average MAE was 0.07 across rapid progressive (0.11), slow progressive (0.09), non-progressive (0.04) and immotile sperm (0.05). Pearson's correlation analysis between manually and AI-predicted motility showed r of 0.67, p<0.001 for rapid

progressive, 0.41, p<0.001 for slow progressive, 0.51, p<0.001 for non-progressive and 0.88, p<0.001 for immotile sperm. The results show that differentiating between rapid progressive and slow progressive motility is difficult, but the model is still able to do this better than the ZeroR baseline, which was 0.15 for rapid progressive and 0.11 for slow progressive. This is interesting since rapid progressive motility has been regarded challenging to assess. The next step would be to compare the results of the algorithm to the human performance.

Limitations, reasons for caution: The sample size is small. The model is based on videos of high quality, and the performance may not transfer well to videos of lower quality. The performance for rapid progressive motility, which may have an important clinical value, has to be improved.

Wider implications of the findings: This CNN model has a potential to assess sperm motility according to WHO guidelines for progressive motility and immotility. The error values for the automatic predictions are low, and the model shows a good performance taking into account that only videos were used to perform the prediction.

Trial registration number: Not applicable

P-105 Clinical validation of mojo AISA, an artificial intelligence robotic CASA system

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Study question: Can a CASA system based on Artificial Intelligence perform as well as manual semen assessment, within the WHO error margins?

Summary answer: The AI-based CASA systems that mimic high quality assessments show great potential for reducing clinical workloads while increasing treatment efficacy.

What is known already: The field of male-factor fertility investigation is still lacking an automated semen analysis system that can be widely clinically adopted. By leveraging state-of-the-art robotics and Artificial Intelligence (AI), it was possible to build mojo AISA which is an AI and robotic platform designed according to WHO recommendation for semen analysis. This system is based on AI software with a unique convolutional neural network (CNN) that detects and measures sperm concentration and motility while ruling out unwanted cells and debris in raw samples.

Study design, size, duration: This study presents and validates the mojo AISA device. A total of 60 patient samples at ANOVA Karolinska University Hospital were collected and results from manual assessment were compared to mojo AISA for concentration and motility. Semen samples were assessed manually (WHO 2010) and concurrently with Mojo AISA. Manual measurements ranged from 1-206M/ml. This study lasted from May 2020 to December 2020 following informed consent and ethics committee practices of ANOVA.

Participants/materials, setting, methods: Sample preparation protocol for mojo AISA consisted of placing two 10ul drops and covering with two 22x22mm coverslip. Manual assessment followed ANOVA EQA procedures akin to the WHO. A CNN was trained using videos captured with mojo AISA as input data. Images were annotated to form a validation set by which the AI was trained. To account for sampling error, videos of Hamilton Thorne Accubeads+ were captured using mojo AISA and the mojo counting chambers.

Main results and the role of chance: Comparing the concentration measured by mojo AISA with the known value for each microbead, results are in agreement of 86%, within the confidence interval of the microbeads. The mean relative error was 6.7% and maximum error was 11%. Therefore, Accubeads+ validation proved no observational error regarding the use of mojo AISA microscope. As for comparing mojo AISA to manual assessment for concentration, Pearson (Spearman) correlation was 0.95 (0.97). The mean relative error was 24.8% and maximum relative error was 71.1%, where 90% of samples were below 50% error. By looking at the concentration range between 10 and 20 M/ml, mojo AISA displayed a mean error of 18.5%. For motility, as comparing mojo AISA to manual assessment, a result of 35.4% mean relative error was obtained. To conclude, mojo's robotic solution shows promise for clinical practice as the AI continues to improve. In 6 months, sperm concentration correlation improved by 3-fold. Next, the AI will be further clinically trained for low concentration.

Limitations, reasons for caution: mojo AISA requires further development, especially for very low concentration ranges, below 5M/ml, due to high sensibility to false positive detections. The same applies to post-vasectomy samples. Additionally, the necessity to compute the motility of each sperm scales poorly with high concentration generating a poor experience for high volume clinics.

Wider implications of the findings: Automation is crucial in several industries. It enables fertility clinics & andrologists to standardize male factor infertility measurements (if paired with widespread standardization of protocols for automation) while enabling them to put more focus on demanding activities of their profession and removes human biases of inter-laboratory performance.

Trial registration number: Not applicable

P-106 The evaluation of dietary score representing the overall effect of men's diet to semen quality on couple's fertility

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Study question: Is men's diet associated with assisted reproductive technology (ART) outcomes?

Summary answer: An empirical dietary score representing the overall effect of men's diet on semen quality was unrelated to ART outcomes.

What is known already: Multiple studies have related various aspects of men's diet to semen quality. Generally, healthier foods, such as fish, vegetables, and fruits, have been related to better semen quality, whereas unhealthy foods, like processed and red meats, have had the opposite relationship. Nevertheless, while bulk semen parameters are important biomarkers of male fertility and a diagnostic cornerstone for male factor infertility, they are imperfect predictors of a couple's fertility.

Study design, size, duration: Couples presenting to the Massachusetts General Hospital Fertility Center between April 2007 and April 2018 were invited to participate in the Environment and Reproductive Health (EARTH) study, a prospective cohort study. Men's diet was assessed with a previously validated food frequency questionnaire. A dietary score reflecting the overall relation of men's food intake with semen quality parameters was empirically derived using reduced rank regression (RRR). The resulting dietary score was related to ART outcomes.

Participants/materials, setting, methods: We used information from 349 men (908 semen samples) to derive the empirical diet pattern and data from 231 couples (407 ART cycles). The primary outcome was the probability of live birth per treatment cycle; secondary outcomes were semen quality, and fertilization, implantation, and clinical pregnancy rates. We evaluated the association between the dietary score and these outcomes using logistic generalized linear mixed models to account for repeated cycles while adjusting for confounders.

Main results and the role of chance: Men had a median baseline age and BMI of 36.8 years and 26.9 kg/m^2, respectively. The empirical diet pattern was significantly associated with all semen parameters. One standard deviation increase in the empirical diet pattern was associated with lower volume (-0.10 standard units [95% CI: -0.17 to -0.04]) and to higher sperm total sperm count (0.13 standard units [0.06 to 0.20]), concentration (0.17 standard units [0.10 to 0.24]), total motility (0.14 standard units [0.07 to 0.20]), progressive motility (0.08 standard units [0.01 to 0.15]), and normal morphology (0.18 standard units [0.11 to 0.25]). Couples with men in the lowest quartile of the empirical score were more likely to have a diagnosis of male infertility than couples with men in the highest quartile (49% vs 24%). Despite the association with semen parameters, the empirical diet score was not related to any clinical outcome of infertility treatment with ART. The adjusted probabilities of implantation, clinical pregnancy and live birth in the lowest and highest quartile of the empirical score were 0.62 (0.50-0.73) and 0.55 (0.45-0.66), 0.57 (0.46-0.69) and 0.50 (0.40-0.61), and 0.49 (0.37-0.62) and 0.36 (0.25-0.48), respectively. Analyses excluding couples with a diagnosis of male factor infertility yielded similar results.

Limitations, reasons for caution: We evaluated the relationship only among couples presenting to a fertility center and therefore it is unclear whether findings can be generalized to couples trying to conceive without ART.
Wider implications of the findings: Given ART is a robust intervention including stringent sperm selection procedures, any effect that empirical diet may have on a couple's chances of conceiving through assisted reproduction is unlikely to reflect the effect of these factors on bulk semen quality parameters.
Trial registration number: The project was funded by ES009718, ES022955, ES026648, and ES000002 from the National Institute of Environmental Health Sciences, and P30DK46200 from the National Institute of Diabetes and Digestive and Kidney Diseases

P-107 Does hematological cancer have the same impact on sperm quality as testicular cancer?

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Study question: Does hematological cancer have the same impact on sperm quality as testicular cancer before chemo or radiotherapy? Summary answer: Hematological cancer has no impact on sperm quality before treatment.
What is known already: The deleterious effects of chemo and radiotherapy on testicular function are well known. Furthermore, testicular cancer causes a negative impact on sperm quality, even before treatment, probably due to local action. Hematological cancer, particularly Hodgkin lymphoma, seems to produce inflammatory alterations in the testis. However, it is not clear if hematological cancer can compromise spermatogenesis, as does testicular cancer.
Study design, size, duration: Observational, cross-sectional, retrospective study using data from 360 patients seen at a private infertility clinic between 1992 and 2019 for sperm cryopreservation before treatment. The data were collected from electronic records in a prospective database.
Participants/materials, setting, methods: Seminal samples from patients that cryopreserved semen due to hematological or testicular cancer were compared. Sperm analyses were performed according to the 2010's World Health Organization (WHO)'s parameters. Seminal volume, total sperm number, sperm concentration, total and progressive motility, and vitality were analysed. In the hematological group, leukemia and lymphoma, and Hodgkin and non-Hodgkin lymphoma were compared. Student t-tests and Chi-Square were used, considering $p<0.05$ statistically significant.
Main results and the role of chance: This study included 295 patients with testicular cancer (TEST) and 100 with hematological cancer (HEMAT). Patients that had already started chemo or radiotherapy (4 HEMAT and 12 TEST) were excluded, and 4 HEMAT and 15 TEST were azoospermic or cryptozoospermic (41.7% vs. 53,0%, p = 0,792). The other parameters were analysed in 92 HEMAT and 268 TEST. The mean age of the HEMAT group was 28.2 years and 27.9 for the TEST group (p = 0,858). The TEST group had higher rates of oligozoospermia (50.7% vs 31.5%, p = 0.001) and of severe oligozoospermia (29.5% vs 15.2%, p = 0.006) than the HEMAT group. Furthermore, 69.6% HEMAT had normal concentration, compared to 45.9% TEST ($p<0.001$). The mean concentration of the HEMAT group was 35 mi/mL, normal according to the WHO's standards, and the TEST group was 12 mi/mL, below the WHO's normal standards ($p<0.001$). No difference was found when comparing leukemia and lymphoma, or Hodgkin and non-Hodgkin lymphoma.
Limitations, reasons for caution: Besides the fact that this study is retrospective, it also has a small sample size. Furthermore, no analyses regarding sperm morphology were made.
Wider implications of the findings: In this study, testicular cancer had a negative impact on spermatogenesis and sperm quality, whereas hematological cancer did not. However, counseling regarding fertility preservation using sperm banking prior to chemo or radiotherapy should be reinforced in all young cancer patients.
Trial registration number: not applicable

P-108 Which infertile men with normal semen analysis deserve a second semen analysis in the real-life setting?

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Study question: Guidelines suggest that one semen analysis is sufficient during the diagnostic work-up of an infertile man in the case of normality as for WHO criteria.
Summary answer: We investigated the rate and the clinical features of men with abnormal sperm parameters at a second test, after a normal first semen analysis.
What is known already: A second test is recommended when the first semen analysis depicted abnormal sperm parameters.
Study design, size, duration: Complete demographic, clinical and laboratory data from 1358 consecutive primary infertile men (infertility as for WHO definition) were analysed. Health-significant comorbidities were scored with the Charlson Comorbidity Index (CCI). Serum hormones were measured in every patient. Patients underwent two consecutive semen analyses at the same laboratory, which followed 2010 WHO reference criteria.
Participants/materials, setting, methods: Descriptive statistics and logistic regression models tested the association between clinical variables and semen parameters. Receiver operative characteristic (ROC) curves were used to assess the relationship between clinical variables and to create a composite risk score for pathological sperm parameters at a second test.
Main results and the role of chance: At first analysis, 212 (15.6%) infertile men had normal semen parameters. Of 212, 87 (41.0%) had a second normal semen analysis, while 80 (37.7%), 35 (16.5%) and 10 (4.7%) men showed 1, 2 and 3 pathological sperm parameters at second test. Men with a pathological second semen analysis had higher CCI scores ($p<0.001$), smaller testicular volume ($p<0.001$) and higher FSH values ($p<0.01$) than those with normal second samples. Overall, despite being within normal ranges, sperm concentration was lower [34 (23-57) vs. 62 (35-94); $p<0.01$] in men with an abnormal second sample compared to those with confirmed normality. At multivariable logistic regression analysis, smaller testicular volume (OR 0.9, p=0.03), FSH (OR 1.2, $p<0.01$), and lower sperm concentration (OR 0.9, $p<0.01$) were associated with pathological second semen analyses, after accounting for age and CCI. ROC curves showed that testicular volume <15 ml, FSH values >6 mUI/ml and sperm concentration <40 mil/ml had good predictive ability for pathologic second sperm parameters (all AUC >0.8). Considering 1-point for each of the previous variables, the chances of a pathological second analysis increased from 38.8% to 74.6%, 77.3% and 100% among patients with risk scores of 0, 1, 2 and 3, respectively ($p<0.001$).
Limitations, reasons for caution: It is a retrospective analysis at a single, tertiary-referral academic centre, thus raising the possibility of selection biases. In spite of this, all patients have been consistently analysed over time with a rigorous follow-up, thus limiting potential heterogeneity in terms of data reporting.
Wider implications of the findings: Approximately 60% of infertile men with a normal semen analysis depicted a pathological second test. Smaller testicles, higher FSH, lower sperm concentrations were independently associated with a pathologic second test. These features could be useful to identify those infertile men with a normal semen analysis who deserve a second test.
Trial registration number: not applicable

P-109 Comparison between the outcome of sperm vitrification protocol and conventional slow freezing protocol for semen cryopreservation

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Study question: Does sperm vitrification technique helps in increasing sperm survival and low DNA fragmentation index post warming.

Summary answer: Sperm vitrification protocol results in better motility, high progression and low DNA fragmentation index as compared to slow freezing.
What is known already: Cryopreservation is ceasing and resuming the cell metabolism, which can be achieved by different techniques like slow freezing and vitrification .Vitrification allows solidification of the cells and extracellular milieu into a glass like state without formation of ice which protects intracellular and extracellular ice formation, and further helps in avoiding different types of cryo-injuries and cellular damage. Study design, size, duration: Comparative study from July 2019 to Oct 2020 in IVF unit of IKDRC Hospital. Two hundred and ten patients were randomized by computer generated list and divided into two groups. Group 1 (n= 110) samples cryopreserved by vitrification and Group 2 (n=100) samples cryopreserved by conventional slow freezing.
Participants/materials, setting, methods: Semen sample were analyzed by WHO 2010 laboratory manual, including all normozoospermic samples , other abnormal samples were excluded from the study . Method of semen preparation before cryopreservation is similar for both the groups, double density gradient method of preparation was used . Semen sample with high viscosity, hypo and hyper-spermia were also excluded. Similar cryovials of 2ml volume were used for both groups.
Main results and the role of chance: In group 1 where samples were cryopreserved by vitrification sperm motility was (54.3 % vs 49.2%)vs in group 2 where samples were cryopreserved by slow freezing , non- significant difference were observed , but progressive motility was significantly higher in group 1 as compared to group 2 (36.8%vs17.9%) and DNA fragmentation index is significantly lower in group 1 vitrification than in group 2slow freezing (9.7% vs 20%).
Limitations, reasons for caution: Technical proficiency of the operator to avoid human errors and still larger randomized control studies are needed to strengthen these results
Wider implications of the findings: Our study demonstrates that vitrification is better than slow freezing of human sperm, improved survival rates with high progression were found with vitrification and low DNA fragmentation index were also observed in samples cryopreserved with vitrification protocol.
Trial registration number: not applicable

P-110 Does the Body Mass Index affect sperm quality?

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Study question: Does the body mass index (BMI) have an impact on semen analysis results?
Summary answer: The increase in BMI has a negative impact on sperm motility.
What is known already: Obesity is an increasingly prevalent health condition worldwide and can affect male fertility in various ways. It is known that obesity can cause testicular inflammation, higher testicular temperature, hypogonadism, sperm DNA fragmentation, and erectile dysfunction. However, there are still conflicting data regarding the correlation between BMI and semen parameters in the seminal analysis.
Study design, size, duration: Observational, cross-sectional, retrospective study using data from 1147 patients seen at a private infertility clinic between 2010 and 2020. The data were collected from electronic records in a prospective database.
Participants/materials, setting, methods: Patients were divided according to BMI (healthy weight, overweight, obesity classes I, II, III), and their seminal profiles were compared, according to 2010's World Health Organization's parameters. Cancer, cryptorchidism, viral orchitis, altered karyotype, Y chromosome microdeletions, vasectomy reversion, and testosterone use were excluding factors. Student t-tests and multiple linear regression were used for statistical analysis. The results were adjusted for age, alcohol, tobacco, and drug use, medication intake, physical activity, comorbidities, and scrotum heat factors.
Main results and the role of chance: From a total of 1384 patients, 219 were excluded. The BMI varied between 18,9 and 50,8 kg/m². From the 1147 patients, 297 had BMI 18.5-24.9 kg/m² (healthy weight, group 1), 611 had BMI 25-29.9 kg/m² (overweight, group 2), 179 had BMI 30-34.5 kg/m² (obese, group 3), 60 had BMI ≥ 35 kg/m² (extremely obese, group 4). The mean age for groups 1 through 4 was 37.6, 38.5, 38.2, and 36.5 years old. The comparison of the groups' seminal parameters shows a significant decrease in progressive and total motility in patients with BMI ≥ 35 kg/m².The progressive motility was 43.8% in group 1, 44.1% in group 2, 42.4% in group 3, and 35.2% in group 4 (p=0.07) and the total motility was 54.4%, 54.1%, 53.6%, and 45.9%, respectively (p=0.012). The complementary analysis determined BMI 29 kg/m² as the cutoff for negative impact on progressive motility (p=0.044) and 31 kg/m² on total motility (p=0.036). The results were still significant after age, use of cannabis, and hypertension adjustments – the other possible interfering factors were not significant.
Limitations, reasons for caution: Besides the fact that this was a retrospective study, it also has a smaller sample size of patients with extreme obesity. This is probably related to the fact that the patients seeking reproductive treatment in a private clinic have a greater purchasing power and lower prevalence of obesity.
Wider implications of the findings: In this study, sperm quality is negatively affected by BMI, with impairment since 29 kg/m² for progressive and 31 kg/m² for total motility. Our data support the potential deleterious role of obesity on semen parameters, reinforcing the importance of weight control in infertility prevention.
Trial registration number: not applicable

P-111 Development of a flow cytometric assay for membrane lipid oxidation in human sperm

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Study question: Is a commercially available lipid peroxidation assay sensitive enough to detect sperm lipid membrane damage and thus provide a novel indicator of male fertility status?
Summary answer: Provisional results demonstrate the novelty of creating a protocol to identify and quantify sperm lipid membrane damage and indicate possible insight into individual male fertility.
What is known already: Cytotoxic lipid aldehydes such as 4-hydroxynonenal (4HNE) created by the damaging effects of reactive oxygen species (ROS) have been studied extensively in sperm, as an indicator of male fertility. This is due to their connection with detrimental effects on sperm function such as morphology, acrosome reactions, motility and fertilization of the oocyte. Although literature states the mechanisms of damage caused to the lipid membrane of the sperm cell, there is no evidence of its quantification or usage as a commercial fertility indicator for human males.
Study design, size, duration: Since the assay is still being developed, there is no formal study size or duration. The goal of this pilot study is to determine whether a commercial lipid peroxidation assay can detect the difference between sperm with high levels of oxidative damage and control sperm cells. We used the remains of sperm samples initially collected for standard semen analysis, which were flash-frozen and then assayed with / without hydrogen peroxide treatment to induce oxidative damage.
Participants/materials, setting, methods: Frozen sperm from consenting donors (n=21) were washed, optionally treated with hydrogen peroxide to induce oxidative damage, stained with a commercially available lipid peroxidation sensor (LPS, Abcam ab243377), and the resulting fluorescence quantitated by flow cytometry. Assay optimization varied the numbers of sperm input to the protocol, the concentration of the peroxidation sensor, the amount and duration of hydrogen peroxide treatment and the effect of paraformaldehyde (PFA) fixation of samples before or after staining.
Main results and the role of chance: Successful detection of lipid damage in control samples

We observed a significant difference at a p-value < 0.05 between untreated samples and all positive controls with hydrogen peroxide concentrations stronger than 500uM ($p < 0.038$) . This indicates that we can detect sperm bearing oxidative damage, and establishes the conditions required to make a positive control sample.

Establishment of assay parameters

Results indicate the concentration of sperm input to the protocol is not a significant factor for concentrations below 5 million/ml. Low concentration samples thus do not require further dilution before testing.

Correlation with DNA damage

A significant direct strong positive Pearson correlation coefficient (R = 0.93, p < 0.023) was found between samples with low DNA fragmentation index (DFI (%), measured by flow cytometric staining with acridine orange) and the LPS flow cytometric data (%).
Limitations, reasons for caution: As yet our data only addresses high level lipid damage induced by peroxide treatment. It remains to be established whether it is possible to detect endogenous LPO damage due to oxidative stress in semen. Future work will correlate our data with motility information and oxidative stress data (measured by MiOXSYS).
Wider implications of the findings: If we are able to develop a direct assay for sperm LPO, this will allow an additional avenue for testing patients with unexplained male infertility, which could in turn affect treatment choices and ART methodology. Improved diagnosis and treatment will potentially improve the lives of families with their fertility matters.
Trial registration number: Not applicable

P-112 Association between dietary total antioxidant capacity and semen quality parameters in male partners of couples attempting fertility

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Study question: Is there any association between dietary total antioxidant capacity (TAC) and semen quality parameters in male partners of couples attempting fertility?
Summary answer: Greater adherence to diets high in TAC was significantly associated with higher total sperm count, sperm concentration, and sperm motility.
What is known already: Among multiple factors that affect the etiology of poor semen quality and male infertility, dietary factors have an important contribution. Also, chronic oxidative stress negatively effects semen quality. Whether adherence to the diet rich in antioxidants is associated with better semen quality remains largely unexplored.The concentration of single antioxidant cannot show the total antioxidant power of the diet, therefore the concept of dietary total antioxidant capacity (TAC) was invented.Dietary scores are useful approach to evaluate the degree of adherence to specific dietary pattern and its benefits in regard to health
Study design, size, duration: This was a cross-sectional study of 350 men from couples attending a fertility center in Tehran, Iran, recruited between June 2015 and September 2019. Men aged 25–50 years with complete dietary data were analyzed.
Participants/materials, setting, methods: Diet was assessed via a reproducible and valid 168-item semi-quantitative food frequency questionnaire to determine the entire antioxidants of the usual diet in order to calculate dietary TAC. Semen quality was assessed according to World Health Organization 2010 guidelines. The association between dietary TAC and semen parameters were assessed adjusting for potential confounders through multiple logistic regression analysis.
Main results and the role of chance: The average age of study participants was 34.9 ± 7.6 years and their BMI was 28.5 ± 4.3 kg/m^2. Participants in the lower tertile of the dietary TAC were lower educated ($p < 0.01$), more physically active ($p < 0.05$), and predominantly had abnormal sperm progressive motility ($p < 0.01$). In the multivariable adjusted models, men in the lowest tertile of the dietary TAC Score had 2.9 times higher likelihood of having abnormal sperm motility, total sperm count and concentration, compared to men in the highest tertile of the TAC score ($p < 0.05$).
Limitations, reasons for caution: The main limitation of our study was its cross-sectional design, limiting our ability to derive causal association.
Wider implications of the findings: Our study suggests that dietary pattern comprising mainly of antioxidant nutrients may help improve semen quality. Our results are consistent with previous studies showing that plant-based diet contains higher levels of antioxidants are associated with better measures of semen quality.
Trial registration number: not applicable

P-113 α-tubulin and tyrosine phosphorylation immunolocalization on human sperm from a globozoospermic patient with proper embryo development after ICSI

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Study question: Can novel sperm biomarkers, such as α-tubulin and tyrosine phosphorylation, help to predict the fertilizing potential osf a globozoospermic sample?
Summary answer: The characterization of α-tubulin and tyrosine phosphorylation provide additional information that could be useful to determine the fertilizing capacity of the globozoospermic samples.
What is known already: Globozoospermia is a severe disorder characterized by the acrosome absence and other sperm alterations such as tail structural disorders and chromatin condensation abnormalities. This set of defects makes globozoospermia correlates with primary infertility and low fertilisation rates. Therefore, additional studies are necessary to know how the globozoospermia affects the disposition of α-tubulin and tyrosine phosphorylation and the relationship between embryo development and pregnancy. In this context, previous studies have independently described different patterns of α-tubulin and tyrosine phosphorylation in normozoospermic samples. Specifically, the continuous α-tubulin distribution along the flagellum and tyrosine phosphorylation have been recently linked to proper sperm functionality.
Study design, size, duration: We conducted a prospective study. The sample was obtained from a globozoospermic patient in October 2019. This sample was divided into two fractions, one proceeded to ICSI and the another was fixed to characterize α-tubulin and tyrosine phosphorylation using confocal microscopy. A total of 200 sperm were analyzed for each biomarker.
Participants/materials, setting, methods: A 38-year-old man requested assisted reproduction in IVF Spain after a failed treatment with no fertilized oocytes. The clinical procedure was performed at IVF Spain and the characterization studies were made at the University of Alicante. The flagellar cytoskeleton was assessed using anti-α-tubulin antibody (Sigma-Aldrich) at a 1:600 dilution. Besides, tyrosine phosphorylation was detected using anti-phosphotyrosine primary antibody (PY20, Sigma-Aldrich) at a 1:500 dilution. Spermatozoa were evaluated using a confocal microscope (Zeiss LSM 800).
Main results and the role of chance: Regarding the α-tubulin characterization, only the 33% of spermatozoa showed continuous labelling in the tail and in the 67% the fluorescence appeared in the terminal piece of the flagellum. Otherwise, we only observed 0.5% of positive tyrosine phosphorylation in the studied cells, whereas the 99.5% of sperm analyzed did not show positive fluorescence. The clinical parameters showed a fertilization rate of 20% with only one embryo after the MII oocyte artificial activation by calcium ionophore. The embryo development was further adequate, and it acquired Bt5AA status on day five. Unfortunately, the pregnancy did not ensue after the embryo transfer.
Limitations, reasons for caution: The main limitation of this study was that due to the very low frequency of globozoospermia (<0.01%) this research was conducted only in one patient. Thus, the present results are preliminary. We need to characterize the aforementioned biomarkers in additional globozoospermic samples to establish relationships with clinical parameters.
Wider implications of the findings: The study of potential candidate biomarkers like α-tubulin and tyrosine phosphorylation in globozoospermia and the linkage with clinical parameters would facilitate the diagnosis and improve the selection of more effective treatment techniques.
Trial registration number: not applicable

P-114 Correlation of GSTM1 polymorphism and oxidative stress with male infertility

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Study question: Is GSTM1 polymorphism a putative biomarker of male infertility. Is there a possible correlation between GSTM1 presence, oxidative stress and male infertility?
Summary answer: A possible correlation may be established between GSTM1 polymorphism, and sperm mobility and morphology. Additionally oxidative potential may also be associated with fertility.
What is known already: Approximately 7% of men worldwide are affected by male infertility, which contributes to 40-50% of all infertility cases. Basic sperm analysis remains the main procedure of diagnosing male infertility, although there is still doubt whether it provides accurate clinical outcomes More accurate tests are essential for the diagnosis of male infertility. Oxidative stress is involved in the etiology of male infertility, with 30% to 80% of infertile men having increased levels of sperm free radicals. Recent research has shown that oxidative stress when combined with GSTM1-null genotype negatively affected the sperm quality of infertility group compared to the control group.
Study design, size, duration: Ninety semen samples were collected and divided into 2 groups. The study group consisted of sperm samples from 51 men identified as infertile according to WHO guidelines(case group).Sperm samples from 39 men with normal sperm count parameters (control group) were used for the control group.
Participants/materials, setting, methods: For all samples a sperm diagram was performed. and DNA was extracted. Polymerase chain reaction with specific for GSTM1 gene primers followed by agarose electrophoresis was applied to detect the presence of polymorphism. The MiOXSYS method was used to detect the oxidative potential.
Main results and the role of chance: This study shows that in the control group the presence of polymorphism was associated with a reduced number of immobile sperm cells (p= 0,035) while it appears to affect the normal morphology of the sperm(p=0,042). In the infertility group the presence of the gene was significantly correlated with age(p = 0.046). No statistically significant difference was observed for the presence of the polymorphism between the 2 groups.In addition, we investigated the effect of oxidative potential with the MiOXSYS system and its relationship with sperm parameters. It was found that the two groups differed significantly when measuring oxidative potential, and that oxidoreduction potential affects sperm concentration/ml, total sperm count, type B motility and viscosity in the infertile male group.
Limitations, reasons for caution: A larger sample size could increase the accuracy of the results.
Wider implications of the findings: Studying the relation of the oxidative stress with sperm parameters may lead to the establishment of a genetic profile of increased risk of infertility, which would be of major importance especially in cases of idiopathic infertility.
Trial registration number: Not applicable

P-115 Supplementation of healthy Sertoli cells into culture media containing follicle stimulating hormone (FSH)/testosterone (T) has no advantage in germ cell maturation

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Study question: In nonobstructive azoospermia (NOA) cases, whether supplementation of healthy Sertoli cells (SCs) has an effect on spermatogenic differentiation in culture medium containing FSH/T.
Summary answer: Expression of Crem and Acrosin increased significantly in both medium with FSH/T and medium with additional healthy SCs but there was no difference between them
What is known already: In NOA the induction of spermatogonial stem cells (SSCs) proliferation and differentiation has been demonstrated using different culture systems. SCs have vital roles in the regulation of spermatogenesis. Hormonal control of spermatogenesis is through FSH and T activity on SCs. Growth factors secreted by SCs via FSH, stimulate proliferation and colonization of SSCs. Although germ cells do not express androgen receptors, FSH receptors are localized on spermatogonia. It is not clear whether native SCs are sufficient for FSH/T added to the culture medium to be effective in induction of spermatogenesis, and whether supplementation of healthy SCs will increase this activity.
Study design, size, duration: 34 NOA and 12 obstructive azoospermia (OA) cases were included. Testicular tissue samples were taken with testicular sperm extraction (TESE) in the study and control groups. In a group of fertile cases, healthy Sertoli cells were identified and purified and then cryopreserved. Tissue samples of each case prepared in standard DMEM/F12 medium were processed in 2 separate environments containing FSH/T and FSH/T plus thawed healthy SCs for 7 days.
Participants/materials, setting, methods: The characterization of healthy SCs isolated from fertile cases was done by flow cytometry (FC) and immunohistochemistry using antibodies specific for GATA4 and vimentin. FITC-conjugated annexin V/PI staining and MTT assay were performed to compare the viability and proliferation of SCs before and after freezing. FC was used to measure the 7th day levels of specific markers expressed in spermatogonia (Vasa), meiotic cells (Crem) and post-meiotic cells (Protamine-2 and Acrosin).
Main results and the role of chance: In Annexin V staining, no difference was found in percentages of live and apoptotic SCs, and MTT exhibited that cryopreservation didn't inhibite the SCs proliferation compared to the pre-freezing state. Vasa and Acrosin basal levels were found to be lower in infertile patients compared to the control group (8.2% *vs.* 30.6% and 12.8% *vs.* 30.5%, $p<0.05$). Compared to day 0 measurements, on the 7th day in FSH/T environment, Crem level increased by 58.8% and Acrosin level increased by 195.5% ($p<0.05$). Similarly, in medium supplemented with healthy SCs, by day 7, the Crem and Acrosin levels were increased to 92.2% and 204.8%, respectively ($p<0.05$). Although Vasa and Protamine levels increased in both groups, they did not reach a significant level. No significant difference was found between the 7th day increase rates of Crem, Vasa, Acrosin and Protamine-2 in either FSH/T-containing medium or in medium additionally supplemented with healthy SCs (58.8% *vs.* 92.2%, 120.6% *vs.* 79.4%, 195.5% *vs.* 204.8% and 232.3% *vs.* 198.4%, respectively, $p>0.05$). Our results suggest that freezing-thawing process would not impair the viability and proliferation of SCs, and adding healthy SCs to the culture medium to correct impaired gene expression does not have an advantage over FSH/T.
Limitations, reasons for caution: The 7-day culture period we determined might be not sufficient for spermatogenic differentiation completion. This period could be extended in order to see further morphological differentiation may need.
Wider implications of the findings: The failure of the culture media containing FSH/T to show the expected effectiveness could be thought to be due to the SCs' inadequate response to these hormones. Therefore, healthy SCs supplementation would be needed, but this could pose ethical issues. Our findings show that it is not necessary.
Trial registration number: 21ÇS532

P-116 A 9-year monocentric retrospective analysis of glutaraldehyde-fixed and semithin section of testicular biopsies and TESE in azoospermic patients

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Study question: What is the outcome of testicular sperm extraction (TESE) after microinjection of frozen–thawed sperm and the correlation with histological analysis in azoospermic patients?
Summary answer: In our cohort of 240 azoospermic patients, sperm could be retrieved in 167 patients (69.6%).
What is known already: Testicular biopsy is a crucial assessment in reproductive practice with diagnostic and prognostic importance for ICSI. Divers histological procedures are used throughout the centres. There is increasing need to accurately analyse histological biopsies in order to characterise different type of spermatogenic failure and allows data storage of value in clinical practice and research. Compared with Bouin's and formalin, glutaraldehyde fixed and semithin section of testicular biopsies have the advantage of yielding not only good cellular morphology but also it allows the possibility of performing electron microscopy.
Study design, size, duration: This is a monocentric retrospective study of TESE practice in azoospermic patients in Strasbourg University Hospital from February 2011 to December 2019.
Participants/materials, setting, methods: A total of 240 azoospermic patients underwent TESE followed by sperm cryopreservation when sperm were present and data of histological analysis and clinical outcome of ICSI were analysed. The analysis include initial hormonal status, type of azoospermia, body max index, classification of histological findings, freezing rate and outcome of ICSI-IVF procedure.
Main results and the role of chance: The mean age of 240 patients was 34.5 years. Out of all patients, 42% were diagnosed with obstructive azoospermia (OA) and 58% patients with non-obstructive azoospermia (NOA). There was no correlation of sperm retrieval with the body mass index. Overall, sperm could be retrieved in 69.6% patients. Spermatozoa were always successfully recovered in patients with normal testicular histological findings, 41.7% patients (n 100). Histological analysis revealed a Sertoli cell-only (SCO) syndrome in 27.5% cases (n 66), hypospermatogenesis in 14.2% (n 34), germ cell arrest in 7.9 (n 19) and mixed pattern in 8.3% (n 20).

In patients with serum FSH concentrations >12 IU/l, 46% of patients (n 42) sperm were present at TESE. In patients with no sperm retrieval, 31.5% had normal FSH levels. Out of all men with elevated FSH, 63% had SCO pattern, 4% germ cell arrest, 21% hypospermatogenesis and 12% mixt patterns. In the group of patients with no sperm retrieved at TESE, histological analysis showed SCO in 69.9% cases, germ cell arrest in 15% and hypospermatogenesis in 9.6% of cases. Out of 167 patients with TESE and sperm cryopreservation, 126 patients undergone ICSI-IVF procedure and 80 babies were born.
Limitations, reasons for caution: This study is a retrospective analysis in a single centre. The cohort was not compared with groups of patients with different histological and fixation techniques.
Wider implications of the findings: Accurate histological diagnosis is a prerequisite for research and clinical data collection and open the possibilities to include patients with NOA in subsequent detailed genetic screen.
Trial registration number: NA

P-117 Bioinformatic analysis of NRF2 in the study of association of NRF2 variant and male infertility related to smoking status

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Study question: Could *Nrf2* polymorphism (-617C>A; rs6721961) and oxidative stress (OS)-induced changes of signature seminal plasma (SP) miRNAs related to Nrf2 provide possible biomarkers of male infertility?
Summary answer: -617C>A SNP is associated with infertility through sperm OS DNA damage and miR-582-5p and miR-20a-5p, differentially represented between spermatozoa of smokers-non-smokers, might regulate Nrf2/ARE axis.
What is known already: As an extrinsic factor causing OS, smoking decreases male infertility by causing sperm membrane damage and DNA fragmentation. Expression of proteins related to the antioxidant defense system and phase 2 detoxifying enzymes controlled mainly by Nrf2/ARE pathway components is vital in managing OS-induced DNA damage. miRNAs, which multiple of are produced abundantly in male germ cells throughout spermatogenesis, have been detected in SP and contribute to multiple biological processes related to male reproductive events. miRNA-expression alterations may be induced in response to OS and without involving DNA sequence changes, miRNAs can provide additional mechanism of regulating the *Nrf2* gene expression.
Study design, size, duration: Wild-type (WT) and SNP (-617) alleles in the *Nrf2* gene were studied in 100 infertile cases and 100 controls and their associations with seminal parameters in relation to smoking status were assessed. In infertile cases, sperm DNA damage level was determined and compared among *Nrf2* genotypes. Interactions between differentially expressed miRNAs (DEMIs) in response to smoking and Nrf2/ARE pathway components were visualized on a miRNA-mRNA regulatory network using CluePedia (v1.5.7) plugin of Cytoscape software (v3.8.2).
Participants/materials, setting, methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was utilized to genotype the *Nrf2* SNP (-617). DNA damages were analyzed by Comet assay. DEMIs were identified by a comprehensive bioinformatics analysis using the miRNA expression dataset GSE44134 downloaded from the GEO database. Predicted targets of DEMIs in smokers were identified by mirDIP portal. Known interactions between Nrf2 and its first neighbors were visualized after selecting STRING-actions, miRTarBase and miRecords validated miRNA source files from CluePedia panel.
Main results and the role of chance: There was significant difference for *Nrf2* polymorphism between fertile and infertile males. The A allele was detected more frequently in the patient group; ($P = 0.001$). The frequencies of the C and A alleles of the *Nrf2* were 62% and 38% in patients, and 78% and 44% in control group. The AA genotype was higher in the infertiles; 14% vs. 3% ($P = 0.001$). In smokers, sperm quality decreased significantly in AA genotype. The risk of DNA damage was highest with 224.58 AU in the AA genotype group, whereas it is the lowest with 164.56 AU in those carrying the CC genotype ($P < 0.005$). 21 differentially expressed miRNAs (including 7 downregulated and 14 upregulated in smokers) were identified. Among the upregulated DEMIs, miR-582-5p, miR-20a-5p, miR-573, miR-186-5p, miR-499a-5p were found to target the *Nrf2* mRNA, suggesting their usage as biomarkers capable of indicating the antioxidant ability of the male reproductive system. The interrelations between Nrf2/Nrf2 direct interactors and DEMIs revealed the regulatory role of hsa-miR-20a-5p in SQSTM1/p62-Keap1-Nrf2 axis linked to selective autophagy. hsa-miR-582-5p was found to regulate the JNK/Jun/caspase-3 pathway, previously shown to be activated in response to OS, in which JUN can activate or suppress the *Nrf2* expression.
Limitations, reasons for caution: Small number of cases while evaluating the effect of smoking weakens our ability to generalize the results. Including other coexisting factors and larger patient groups carrying other functional variants of *Nrf2* as well as confirming the results at the protein level would further strengthen the results of the study.
Wider implications of the findings: This study is the first to report -617C>A polymorphism in the *Nrf2* gene in the Turkish population and such a SNP may cause impaired fertility in men, especially in smokers, through oxidative metabolism. Considering these data may be valuable in determining risk groups.
Trial registration number: N/A

P-118 A sperm selection technique to mitigate paternal contributions to embryo aneuploidy

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Study question: Can microfluidic sperm selection (MFSS) select male gametes without sperm chromatin fragmentation (SCF) and double-stranded DNA breaks (dsDNA) in order to generate euploid conceptuses?
Summary answer: Couples treated by ICSI with MFSS had significantly improved embryo ploidy rates and pregnancy outcomes, demonstrating the efficacy of this novel selection method.
What is known already: SCF has been linked to infertility, specifically to embryo developmental and implantation failure. This damage can be both single-stranded (ssDNA) or double-stranded (dsDNA). Recent studies have shown that dsDNA in particular causes chromosomal aberrations and contributes to embryo aneuploidy, which leads to implantation failure.

Study design, size, duration: Consenting couples treated at our center by intracytoplasmic sperm injection (ICSI) with spermatozoa selected by MFSS were included. The majority of these couples had a medical history significant for elevated SCF, recurrent implantation failure, and embryo aneuploidy. ICSI clinical outcome, as well as preimplantation genetic testing for aneuploidy (PGT-A) and frozen embryo transfer (FET), was recorded and compared to the couples' historical treatments following sperm selection by density gradient centrifugation (DGC).
Participants/materials, setting, methods: From 2016 to 2020, 51 consenting men had their ejaculates screened for SCF levels by terminal deoxynucleotidyl dUTP transferase nick-end labeling (TUNEL) using a commercially available kit. At least 500 spermatozoa were assessed per patient, with a normal threshold of ≤15%.

To screen for dsDNA, neutral Comet using a modified in-house protocol was also performed in a pilot study. At least 200 spermatozoa were assessed per patient, with a normal threshold of ≤3%.
Main results and the role of chance: A total of 51 men (average age, 41.0±8 years) had mean sperm concentrations of 39.0±33x106/mL, 38.4±12% motility, and 2.1±1% normal morphology. Following DGC and MFSS, the concentrations were 4.7±8 and 4.3±8x106/mL and the motility was 64.0±33 and 98.0±3%, respectively (P<0.0001). The average SCF decreased from 20.1±18% in the ejaculate to 16±3% following DGC, but was 2.9±4% after MFSS. The dsDNA fell from 3.4±3% in raw specimens to 2.9±1% after DGC, and to only 0.5±0.7% following MFSS (P<0.0001).

These men underwent ICSI with their female partners (average age, 37.3±4 years), with sperm selected by DGC; they achieved a fertilization rate of 56.4% (337/597) with 26.0% euploid embryos (36/139). FET cycles from these embryos yielded an implantation rate of 8.3% (2/24) and a clinical pregnancy rate (CPR) of 15.4% (2/13), but both miscarried.

These couples then underwent ICSI with MFSS, with a fertilization rate of 78.0% (588/754; P<0.0001) and 50.0% (172/344; P<0.0001) euploid embryos after PGT-A. A total of 37 embryos have been replaced, with an implantation rate of 67.6% (25/37; P<0.0001) and a CPR of 73.5% (25/34; P<0.001), with an ongoing/delivery rate of 70.6% (24/34; P<0.0001).
Limitations, reasons for caution: While the oocyte contribution cannot be discounted, MFSS was able to yield spermatozoa that had the highest motility and ability to produce euploid embryos following ICSI.
Wider implications of the findings: The genome and epigenome of the spermatozoon, and their contribution to reproductive outcomes, are being vigorously explored and scrutinized. Alternative approaches to gamete selection, such as MFSS, in couples with elevated SCF and dsDNA provide the best chances for future pregnancies by mitigating embryo aneuploidy.
Trial registration number: not applicable

P-119 The correlation between sperm chromatin fragmentation and intrauterine insemination outcome

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Study question: Does sperm genomic integrity affect the intrauterine insemination (IUI) outcomes in couples with unexplained infertility and young maternal age?
Summary answer: Spermatozoa with higher genomic integrity are correlated with higher clinical pregnancy rates in couples with unexplained infertility undergoing IUI.
What is known already: It is known that elevated sperm chromatin fragmentation (SCF) on the male gamete affects embryo development and implantation. This is particularly relevant in IVF as well as programmed intercourse and IUI. By complementing the standard semen analysis with an SCF assay, we can assess the competence of the male gamete and its ability to generate euploid embryos and healthy offspring. Elevated SCF has been used as a way to identify subtle male factor infertility in couples undergoing IUI with poor pregnancy outcomes in order to plan for further treatments.
Study design, size, duration: This is a retrospective cohort study of IUI outcomes of couples with young maternal age and a negative infertility workup treated at our center from 2016–2020. Terminal deoxynucleotidyl dUTP transferase nick-end labeling (TUNEL) assay was used to assess sperm genomic integrity. Couples were grouped based on SCF level: normal (≤15%) or abnormal (>15%). Rates of clinical pregnancy, defined as the presence of a fetal heartbeat, were compared between the groups following IUI.
Participants/materials, setting, methods: A total of 189 consenting couples, in which the female partner had a normal uterine cavity and patent fallopian tubes, underwent 454 IUI attempts. Only women ≤37 years old were included to control for age-related confounding factors. At least 500 spermatozoa were assessed per patient, and a threshold of ≤15% was considered normal. Women were either untreated for natural cycle IUI or stimulated with clomiphene citrate, gonadotropins, or Letrozole.
Main results and the role of chance: A total of 454 IUI cycles were reported at our center; 302 of these were carried out in 132 couples in which the male partner had normal SCF averaged at 9.29%. The average maternal age was 34.1±3 years, and the average paternal age was 37.1±5 years. These men had the following semen parameters: a concentration of 46.2±5x106/mL, 43.8±3% motility, and an average SCF of 9.3±3%. There were 45 documented clinical pregnancies (45/302, 14.9%) as confirmed by the presence of at least one fetal heartbeat detected by ultrasound; 26 delivered, 9 are ongoing, 5 were spontaneous abortions, and 5 were lost to follow-up. A total of 57 couples in which the male partner (37.2±5.9 years) had abnormal SCF underwent 152 IUI cycles (maternal age, 34.0±2.7 years). The men had the following semen parameters: an average SCF of 23.8±10 (p<0.0001), a concentration of 26.0±10 x106/ml, and 40.1±4% motility. These IUI attempts yielded a clinical pregnancy rate of only 4.6% (7/152; P<0.0001); 4 delivered and 3 were spontaneous abortions.
Limitations, reasons for caution: This study is a retrospective cohort analysis of a relatively small number of patients. Furthermore, most patients were screened for SCF due to at least one prior IUI failure. A prospective, randomized trial, in which men are concurrently screened for SCF levels at the first IUI attempt, would be ideal.
Wider implications of the findings: Assessment of SCF at the initial male infertility screening can be a useful tool to investigate the competence of the male gamete. Screening couples with idiopathic infertility for a subtle male factor would guide those with higher SCF toward alternative reproductive treatments to avoid unnecessary IUI treatments.
Trial registration number: Not applicable

P-120 Selecting spermatozoa with the highest genomic integrity in order to enhance clinical outcomes in men with high DNA fragmentation levels

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Study question: What are the best methods of selecting spermatozoa with the highest genomic integrity in order to improve embryo implantation and term pregnancy rates with ICSI?
Summary answer: Testicular or ejaculate spermatozoa isolated by microfluidic sperm selection (MFSS) were characterized by superior genomic integrity with improved clinical pregnancy and delivery rates.
What is known already: In couples with unexplained infertility, a subtle male factor can often be identified. Both single-strand (ss) and double-strand (ds) DNA nicks and breaks hinder the ability of the male gamete to support embryonic development. Surgical retrieval of spermatozoa from the proximal male genital tract can prevent their exposure to oxidative stress. Moreover, use of membrane-based microfluidics chips has been shown to allow for selection of the most progressively motile spermatozoa with higher genomic integrity.
Study design, size, duration: Over the course of 48 months, 86 consenting men presenting with high sperm chromatin fragmentation (SCF) in their ejaculate with prior ART failure underwent a subsequent cycle with specimens retrieved by testicular biopsy or ejaculate processed by MFSS. A concurrent TUNEL assay was performed on samples collected or selected by each method. Sperm specimens of both origins were utilized for ICSI cycles. Semen parameters, chromatin integrity, and pregnancy outcomes were compared between the two methods.
Participants/materials, setting, methods: Fresh ejaculates from consenting men were collected for standard semen analysis (WHO 2010). Testicular biopsy and MFSS were used to isolate spermatozoa with a higher genomic integrity after previous ART failure. SCF was assessed by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) on at least 500 spermatozoa under a

fluorescent microscope with a threshold of ≥15%. MFSS was carried out by Zymot® chips. ICSI was performed in the standard fashion.

Main results and the role of chance: A total of 86 men (36.5±5 years) had the following semen parameters: volume of 2.6 ±1mL, concentration of 27.0±33 x 106/mL, 35.6±15% motility, and high SCF (24.1±10%). They underwent 146 ICSI cycles with their partners (maternal age, 33.7±3) resulting in a high incidence of pregnancy loss (100%; 13/13). Of those who failed to conceive, 22 couples used surgically retrieved spermatozoa (SRS) with a concentration of 1.8 ± 4 x 106/mL (*P*<0.01), 5.0±11% motility (*P*<0.01), and an SCF of 12.6 ± 6% (*P*<0.0001). SRS was used in 37 ICSI cycles, yielding a fertilization rate of 61.6% (204/331, *P*<0.01), an implantation rate of 10.6% (9/85, *P*<0.01), a CPR of 23.5% (8/34, *P*<0.01), and a delivery rate of 17.6% (6/34, *P*<0.01). Another 24 couples underwent ICSI cycles with ejaculated spermatozoa processed by MFSS with a concentration of 1.8±3 x 106/mL *(P*<0.01), but an increased motility of 99±1% (*P*<0.01) and an SCF of 1.2 ±1%, lower than both the raw and testicular specimens (*P*<0.0001). MFSS-processed specimens resulted in a fertilization rate of 76% (335/441, *P*<0.01), an implantation rate of 26.3% (15/57, *P*<0.05), and a CPR of 67.9% (19/28, *P*<0.01), of which 15 patients delivered and 2 pregnancies are ongoing (89.5%; *P*<0.01).

Limitations, reasons for caution: This is a preliminary study on a small number of subjects. A randomized prospective study conducted on a larger cohort would be required to confirm our findings.

Wider implications of the findings: SCF severely affects pregnancy by impairing embryonic development, consequently promoting implantation failure. While retrieving spermatozoa from the germinal epithelium is a viable option, MFSS provides an alternative. Although MFSS requires an adequate number of sperm with good kinetic characteristics, it provides a more palatable option, reducing surgical risk and costs.

Trial registration number: Not Applicable

P-121 Selection and separation of X- and Y- chromosome-bearing mammalian sperm using SPERMAN technology

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Study question: How can we select and separate X and Y-chromosome bearing sperm cells without causing cellular damage and reduced fertility?

Summary answer: AI powered SPERMAN technology can sort X and Y-chromosome bearing sperm populations without using harmful fluorescent dyes and lasers.

What is known already: Most common and reliable method to sex sorting (separation of X and Y- chromosome bearing sperm cells) is by using fluorescence activated cell sorting (FACS) which takes advantage of the difference of amount of DNA in X and Y-chromosomes. Unfortunately this method causes reduced fertility and cellular damages due to the lasers and fluorescent dyes that are used. There are new and experimental developments in sex-sorting such as using immunological approaches to separate sperm cells. Current sorting method damages sperm cells. However, there is no automated and easy to use sperm sorting technology available.

Study design, size, duration: In this study, we have compared the quality parameters (motility, viability, AR status, DNA packaging) of sorted sperm cells to unsorted sperm cells. We have used epididymal sperm samples from 10 C57BL/6 mice (Charles River) and frozen semen samples from 6 Holstein bulls (Sexing Technologies) and frozen semen samples from 8 human sperm donors (Fairfax Cryobank). Samples were divided into two groups: sex-sorted and unsorted sperm.

Participants/materials, setting, methods: Sperm samples from 8-week-old C57BL/6 mice were collected from epididymal region, frozen bull semen samples and human semen samples were thawed at 37°C then separated into two groups as sorted and unsorted sperm. Sorted samples in modified SP-TALP medium were loaded on the microfluidic chip of Sperman device, after the 30min long sorting process ended, all samples were centrifuged gently at 100g for 5 min, quality parameters were measured by using CASA and FACS.

Main results and the role of chance: Sperman device sorted X-bearing sperm cells at 81% purity and Y-bearing cells at 73% in mouse samples. Sperman device was able to sort X-bearing sperm cells at 78% purity and Y-bearing cells at 70% in bull samples.S perman device sorted X-bearing sperm cells at 85% purity and Y-bearing cells at 76% in human samples.

Our study shows that in mouse, bull and human sperm samples, sperm DNA quality, sperm concentration, progressive motility and AR status results from the sorting with SPERMAN device are comparable with the unsorted samples. For sperm DNA quality, both the Spearman rank correlation coefficient and concordance correlation coefficient are above 0.97, indicating a high agreement between the unsorted samples and SPERMAN sorted samples.

Limitations, reasons for caution: In this study we only compared sperm quality parameters between unsorted sperm cells and Sperman-sorted sperm cells. Ideally a follow up study would be to look in detail at fertilization success rates and embryo growth. Also, genomic and proteomic profiles of unsorted and sorted sperm cells could be different.

Wider implications of the findings: In livestock, sperm sex sorting has high value in terms of economic impact on the livestock management. Unfortunately, there is one expensive single technology dominates the sex-sorting market causing high fees poor outcomes. We believe that Sperman technology provides farmers more cheaper and better sex-sorting technology.

Trial registration number: not applicable

P-122 Benefit of use of theophilline in ICSI with testicular sperm

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Study question: Would the use of theophylline have an effect on ICSI outcomes?

Summary answer: The cumulative pregnancy rate after the transfer of fresh and frozen embryos (FET) becomes more important justifying the addition of theophylline as an efficacy variable.

What is known already: Absolute immotile spermatozoa is one of the most important causes of reduced fertilization and pregnancy rates after ICSI, immotility of testicular spermatozoa is a physiological event resulting from metabolic sperm immaturity. Over the years, there have been numerous attempts to resolve this problem by identification of pharmacological agents that might improve sperm motility. In particular, theophylline turned out to be an effective tool in stimulating motility in human semen and identifying viable sperm in testicular sperm extraction sperm (TESE) patients. aim of this study is to evaluate ICSI outcomes after the use of theophylline to select viable spermatozoa

Study design, size, duration: This prospective, comparative randomised study was conducted in Fertillia ART center in Tunisia, between november 2017 to november 2020. All patients underwent ICSI cycles with testicular sperm were included. The exclusion criteria consist of cycles with female partner age >42 years and/or cycles with no information about pregnancy outcomes. In our study, the cycles were categorized into two groups according to sperm selection method used in ICSI procdure.

Participants/materials, setting, methods: The present study include 678 cycles. This cohort was randomly divided into two groups according to method of spermatozoa selection. Treatements arms were performed by theophylline to improve sperm motility (Group A) and the Hyposmotic swelling (HOS) test to indicate sperm vitality (Group B). Clinical and biological parameters, the duration of sperm selection and ICSI outcomes were compared between the two groups. A statistical significant difference was accepted when the p value was <0.05.

Main results and the role of chance: Baseline clinical parameters were found to be comparable in the two groups. No differences regarding number of oocytes retrieved or MII oocytes were reported . he sperm selection was easier in the group A (time interval : 12±7,2 minutes) than group B (time interval : 22±10 minutes) (p<0,05). No significative difference was observed between goups in the Fertilization rate (group A : 66% vs group B : 68%) ; Cleavage rate (group A : 76.20% vs. Group B : 75.56%) and Blasulation rate (group A : 63.60% vs. Group B 60.61%) p>0 ,05. The Cumulative Pregnancy rate for group A (31. 5%) was higher than group B (30%, p>0 ,05). Indeed, The rate of frozen cycles is significantly higher for group A compared to group B (30% vs. 4.5% p<0,05).

Limitations, reasons for caution: Need large sample size.
Wider implications of the findings: Theophylline reduces significantly the time needed for sperm selection, Which positively affects the ICSI result.
Trial registration number: Not applicable

P-123 How to develop accurate Computer Assisted Sperm Analysis (CASA) AI in the absence of protocol standardization and abundance of human error when performing semen analyses?

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Study question: How can an automation & artificial intelligent tools be developed to perform according to WHO recommendations?
Summary answer: Developing CASA performs at < 20% error margin requires AI trained with high quality datasets and a robotic system adheres to WHO guidelines.
What is known already: A survey of 40 andrology laboratories, in 22 countries, revealed that > 90% had nonconformities in correct use of equipment, standardisation of protocols and quality control, leading to a lack of compliance to WHO protocols. Conventional CASA systems can standardize analysis, but controversy has occurred due to differences between manual and automated analyses stemming from: 1) all cells in a semen sample are detected including debris; 2) protocol variation when compared to top-notch manual analysis. The first point can be addressed by AI. The second point can be addressed by robotics designed to adhere to WHO guidelines.
Study design, size, duration: A mojo AISA (AI-powered semen analysis) system was placed in four clinical laboratories mentioned above capturing images of over 300 samples, one million images were generated over a course of 2 years. Mojo AISA's AI was trained on data collected from the four clinics using robotic system is developed according to WHO guidelines.
Participants/materials, setting, methods: For an AI to detect sperm accurately, sperm samples were captured using mojo AISA smart microscopy and then the extracted sperm images expertly annotated. To evaluate the system-ability for semen analysis, fresh sample were analysed for concentration and motility by a manual operator and compared to a mojo AISA test.
Main results and the role of chance: To train the sperm detection AI, representative sperm images were carefully captured using mojo AISA and processed according to the following criteria:

- the number of images and videos to train and to test the model: 50,000 spermatozoon head and tails with various variations
- the variety of images: data used to train the AI has to be representative of the population that will undergo the analysis: 1) wide concentration ranges from 0 to 300 M/ml, 2) high and low density of debris and cells, 3) Presence of slight aggregations
- careful and precise annotation: expert andrology scientists annotated sperm images and identify objects to exclude, such as debris in seminal plasma, Mojo AISA is an attempt strictly build CASA AI system to WHO-guidelines. The marriage of AI and robotics automation has shown a promising results to mimic humans when measuring a semen sample and attempt to obtain results comparable to the manual analysis.

mojo AISA's performance improved three-fold (from 0,85 to 0,95 Pearson sperm count correlation and from >100% means relative error to 25% mean relative error).
Limitations, reasons for caution: Lack of standardization for semen analysis laboratory process globally is a bottleneck towards building a robust multi-center study, on-site CASA testing and generating an actionable data pool for studying the causes behind male fertility declineWider implications of the findings: Key learnings for parties advancing developing AI based on images and videos for application in the fertility space.
Trial registration number: not applicable

P-124 The beneficial effects of novel quercetin delivery system (loaded on bigel) on male fertility parameters of Non-alcoholic fatty liver model: an in-vivo (rat model) study

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Study question: Dose quercetin encapsulated in a bigel slow- release delivery system improve male fertility parameters in Non-alcoholic fatty liver (NAFLD) model
Summary answer: Quercetin in a bigel slow- release delivery system can boost semen parameters in NAFLD rat model
What is known already: Recent molecular and physiological studies have shown that adverse effects of NAFLD extend far beyond the liver. NAFLD can impair male reproductive function by increasing Reactive Oxidative Stress (ROS) levels, reducing the expression of antioxidant genes and inducing damage in testes immune privilege.

Antioxidant therapy and its effectiveness depend on whether the exogenous antioxidant will be readily absorbed to reach high enough that are required to decrease the pathological damages.

Quercetin, as an antioxidant, is able to ameliorate oxidative stress. The design of new drug delivery systems using encapsulating antioxidant can boost its durability and effectiveness.
Study design, size, duration: Bigels were prepared using cottonseed oil/cannabis oil/alginate/ferula gum. Sprague-Dawley rats are housed for 2 weeks, then NAFLD was induced by 58% of dietary calorie as lard and 42 g/L fructose for 16 weeks. The experimental protocol was approved by the ethical committee of Zanjan University of Medical Sciences, Zanjan, Iran.
Participants/materials, setting, methods: After confirming the NAFLD induction, animals divided into five groups: Control, control NAFLD, received 2 mg/kg Quer loaded on bigels, free bigels, free Quer for 45 days as daily gavage. Semen parameters (count, motility, and morphology), viability (Eosin-nigrosine staining) and serum testosterone levels were analyzed. In addition, histological sections of testicular tissues were investigated by Hematoxylin-Eosin (H&E) staining method. In situ detection of apoptosis was performed using terminal deoxynucleotidyl-transferase dUTP nick end labeling (TUNEL) assay.
Main results and the role of chance: The sperm count, sperm motility, normal morphology and testosterone level were significantly lower in the NAFLD group than those the controls. Moreover, higher head and tail abnormality percentages were seen in the sperm of these groups. Bigel-Quer significantly improved the serum testosterone level, sperm count, motility, and morphology compared with the NAFLD group. Spermatogenic cells in all stages of differentiation (spermatogonia, primary spermatocytes, early spermatids, late spermatids) are observed and preserved normally in the testicular tubules and lumen filled with mature sperms in the control group. Interestingly, atrophic changes in the testicular tubule architecture with swelling in spermatogonia cells, detachment from tubule membrane, reduced number of mature sperm, and reduced lumen thickness were seen in the NAFLD. In the Quer, bigel and bigel-Quer-treated groups, swelling and vacuolation rate of germ cells decreased. The testicular morphology, and tubule structure were significantly normalized, especially in the bigel-Quer-treated group. Serum testosterone levels significantly increased and reached the healthy control group in the bigel-Quer group. TUNEL-positive cells in testes increased significantly after NAFLD induction. Quantitative analysis showed a significant decrease in testicular TUNEL-positive cells following bigel-Quer treatment, but not in other groups.
Limitations, reasons for caution: Keeping and daily handling of animals for long-time in animal house for diet-induced NAFLD. NAFLD requires long

periods of treatment to get the desired outcome especially in the case of sperm parameters investigation.
Wider implications of the findings: The bigel showed synergistic effects with Quer for treating infertility in male rats with NAFLD. Stability and bio-availability of Quer are important aspects that should be considered to justify its supplementation. Empowering antioxidant shield of NAFLD patients by Quer supplementation can improve various damage effects and clinical status of diseases.
Trial registration number: Not Applicable

P-125 Evaluation of N-acetylcysteine (NAC) effect on in vitro culture of immature mouse testis following vitrification

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Study question: Can the Culture of cryopreserved immature mouse testicular tissue in the presence of NAC improves the developmental process and prevent apoptosis induction during the culture?
Summary answer: An appropriate dosage of NAC in the culture medium of immature mouse testicular tissue was associated with increased cell survival and spermatogonia stem cell regeneration.
What is known already: Spermatogonial stem cells (SSCs) are the most advanced type of stem cells in the testes of prepubertal boys which are the main targets of oncological treatments. Therefore, the only possible alternative to maintain fertility in prepubertal boys is to preserve SSCs before their depletion by cryopreserving the testicular tissue. Despite the possibility of obtaining viable spermatozoa using cryopreserved testicular tissue cultivated in vitro,cryopreservation methods and damages caused by the culture procedure would be obstacles for maintaining the testicular tissueand it seems that optimizing the culture condition is vital.
Study design, size, duration: Testis tissues were harvested from 6-days-old immature NMRI male mice (n=100) after cervical dislocation and vitrified. After 3 days testicular biopsies were warmed and distributed into control, culture I (not supplemented with NAC) and culture II (supplemented with NAC) groups. To determine the appropriate NAC concentration 8 different dosages of NAC were evaluated in terms of cell survival and the best dose, a culture medium containing 125mmol/L NAC was selected to continue the study.
Participants/materials, setting, methods: Vitrified-warmed fragments (2mm3) obtaining from immature NMRI mice were cultured in vitro for 7 days on agar gel. The effects of culture conditions were assessed by Morphological evaluation of seminiferous tubules (using Hematoxylin-eosin staining). Cell viability, protein expression (caspase-3), and gene expression (Bax, Bcl2, Caspase-3, plzf) were evaluated by flow cytometry, immunofluorescence staining, and real time polymerase chain reaction respectively. Additionally, Malondialdehyde (MDA) concentration in the culture medium was measured by MAD Assay Kit.
Main results and the role of chance: Significant ($p<0.01$) increase in cell viability was observed in the culture II group after 7 days of culture compared to the culture I. Bax/Bcl2 ratio was significantly ($p<0.01$) lower in the culture II group compared to the control and culture I group. The expression of caspase-3 showed a significant ($p<0.001$) increase in the culture II group while immunofluorescence analysis showed low expression of it in all groups. These results were consistent with the high level of Bcl2expression that inhibited Caspase-3 expression and consequently the inhibition of apoptosis, and on the other hand, the presence of NAC showed that plzf expressions significantly ($p<0.001$) increased in culture II group compared to the control and culture I group. Although the presence of NAC did not inhibit all the deleterious effects of culture medium on tissue morphology, NAC was able to maintain better integrity of tissue and seminiferous tubules within central regions compared to the group without NAC. The decrease in MDA level in the presence of NAC (culture II) was also a good indicator to confirm the desired results obtained from the presence of NAC in the culture medium.
Limitations, reasons for caution: Although the findings of the study were satisfactory in mice tissue after 1 week of culture, it is essential to replicate the experiments using human tissue and evaluate the quality and reproductive potential of surviving spermatogonia after long-term storage to become clinically applicable.
Wider implications of the findings: This study highlights the necessity for further experiments to improve the testicular tissue culture conditions for better spermatogonial survival and differentiation to sperm, as the prepubertal fertility restoration methods are promising to be implemented in the clinic in the near future.
Trial registration number: Not Applicable

P-126 A comparison of the efficacy of sperm freezing using high security tubes versus high security straws

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Study question: Are there any advantages in using High security tubes rather than High Security straws for conventional slow sperm freezing?
Summary answer: Freezing sperm in High Security tubes (HST) improved post-thaw recovery rate and motility, and also reduced processing and handling compared to High Security straws (HSS).
What is known already: The use of High Security freezing consumables (HSFC) in an IVF setting is a safe and effective way of eliminating concerns related to viral cross-contamination during storage. The lower diameter of HSS does make them susceptible to warming during handling. The HSFC used in this study is the only CE marked products that are made of resin, leak-proof and shatter-proof in all cryogenic temperatures even in LN2. No previous studies have compared the use of HST with HSS for conventional human sperm freezing. This study sets out to investigate the performance of HST compared to HSS.
Study design, size, duration: The study was designed as a controlled split-sample study with blind post-thaw analysis. Following the routine WHO analysis of 20 semen samples, the remainder of each of the samples was evenly divided and cryopreserved by conventional slow freezing in each of the two different HSFC. The freeze was conducted simultaneously by the same practitioner, employing the same freezing protocol and cryoprotectant. The pre-freeze and post-thaw concentration, total and progressive sperm motility were recorded.
Participants/materials, setting, methods: At one IVF clinic, semen samples with sperm density ≥15million/ml, ≥40% motility, ≥1.5ml were included. Cryoprotectant (SpermFreeze, Fertipro) was added dropwise to unprepared semen and kept at room temperature for 10 minutes before loading into HSFC (0.5ml CBS™HSS; CBS™HST). HSFC were heat-sealed (SYMS; SYMSIII sealers) and placed in vapour for 30 minutes before plunging into LN2. Samples were thawed by immersion in a 37C° water bath for 5 minutes and analysed using WHO methods.
Main results and the role of chance: Paired-t test was used to compare the percentage motility between the different HSFC. All analysis was considered statistically significant when $p<0.01$.

We demonstrated that the sperm recovery rate (Percentage total motility post-thaw/ Percentage total motility pre-freeze) in HST was 66.63 ± 14.94 (mean ± standard deviation) compared to 40.80 ± 14.69 in HSS.

In the HSS, the percentage post-thaw total motility was 19.99 ± 7.21 and the percentage post-thaw progressive motility was 12.26 ± 2.59. In the HST, the percentage post-thaw total motility was 32.57 ± 8.33 and the percentage post-thaw progressive motility was 23.08 ± 5.53. The overall improvement when using HST against HSS was 12.53 ± 5.69, 10.44 ± 5.29 for the total motility and the progressive motility respectively.

Comments were recorded regarding the handling and the condition of the HSS and HST for each freeze event. Neither device displayed any leakage of LN2 or any explosion during the warming. The freezing process was easier and faster using HST rather than HSS. It was also noted that the entire sample can be recovered from the HST, unlike the HSS.
Limitations, reasons for caution: The study looked at sperm recovery in terms of motility only. DNA damage was not considered as a parameter of sperm quality. Also, fertilization, pregnancy rates, live birth rates and the use of poorer quality sperm samples have not been investigated.
Wider implications of the findings: For conventional sperm freezing, the use of HST resulted in improved sperm motility and progression post-thaw, when compared to HSS. This finding supports the use of HST to improve the post thaw quality of sperm, benefitting patients with own frozen samples, recipients of donor sperm and donor sperm banks.
Trial registration number: Not applicable.

P-127 Does sperm quality really matter for conceiving through an intrauterine insemination program? A retrospective analysis of 5920 attempts taking into account repetition of cycles

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Study question: Are parameters of sperm quality part of the prognosis factors for an infertile couple to obtain a live birth when entering an intrauterine insemination program considering repetition of attempts?
Summary answer: Paradoxically, a lower sperm morphology independently predict quick live birth through IUI, as well as younger female age, lower D3 FSH, and higher triggering estradiol.
What is known already: Many studies have highlighted different prognosis factors for obtaining a live birth after IUI involving:

- male parameters (semen),
- female parameters (age, parity, ovulation, tubal, and endometriosis status, history of pelvic surgery),
- couple parameters (duration of infertility, number of previous attempt),
- IUI parameters (follicle number, endometrial thickness, estradiol at triggering, day of IUI, number of spermatozoa inseminated).

However, most of these studies have included small number of attempts, semen parameters were either not collected or assessed with heterogeneity, and repetition of attempts (although iconic for IUI) was not considered, allocating inappropriate weight to cycles which failed in conceiving.
Study design, size, duration: We retrospectively studied the entire cohort of IUI attempts carried out with partner's sperm at our center between 09/09/2003 and 01/17/2017. We included all male, female, couple and IUI parameters available. Each basic semen analyzes included have been carried out by a restricted number of skilled andrologists from our center. The closest semen assessment performed before IUI was considered as male parameter. IUI attempts were considered repeated unless a live birth or IUI abandonment occurred.
Participants/materials, setting, methods: Our primary outcome was live birth occurrence. We included 2228 couples having performed 5920 IUI attempts, with 636 live births obtained. A mixed logistic regression model was used to take into account IUI repetition before obtaining a live birth. A survival analysis using Cox model, with IUI rank as time variable, live birth as endpoint, and taking into account recurrences was carried out to determine which parameter best predict a quick live birth through IUI.
Main results and the role of chance: Included women were 33.7 ± 4.6 years old in mean. Baseline semen assessment was available for 64% of couples.
Muti-variate analysis showed that live birth was more frequent when:

- Femal factors: age was young (33 to 38yo, OR 0.76 [0.60;0.96], >38 yo OR 0.49 [0.35;0.67], p=0.0001), FSH ≤ 8.0 (OR 0.59 [0.45;0.79], p=0.0002), AMH > 8.9ng/mL (OR 0.59 [0.45;0.79], p=0.0001), endometriosis was absent (OR 0.56 [0.36;0.88], p=0.0109), the patient already delivered (OR 1.36 [1.06;1.74], p=0.0034)
- Male factors: sperm motility ≤26.0% (OR 0.71 [0.53;0.96], p=0.0062), sperm vitality ≤72.0% (OR 0.65 [0.47;0.90], p=0.0032), sperm typical form ≤ 25.0% (OR 0.51 [0.34 ; 0.78], p=0.0016),
- IUI attempt factors: total dose of gonadotropin > 495.0 (OR 1.54 [1.26;1.88], p=0.0001) follicle number > 2 (OR 1.23 [1.69;2.21], p=0.0296), Estradiol at trigerring > 215.0pg/ml (OR 1.90 [1.53;2.36], p=0.0001), Endometrial thickness >9.6mm (OR 1.43, p=0.0024), day of IUI >13 (1.53 [1.24;1.89], p=0.0001).

Using Cox model, couples obtained quickly a livebirth if:

- woman age was below 33yo: For 33 to 38 yo, OR 0.37 [0.25;0.54], p=0.0001, >38yo OR 0.19 [0.11;0.32], p=0.0001
- D3 FSH<8 (if above, OR 0.55 [0.34;0.90], p=0.0160) - Sperm typical form ≤25.0 (if above, OR 0.34 [0.20;0.58], p=0.0001)
- Triggering estradiol >215.0pg/ml, OR 1.99 [1.51;2.63], p=0.0001)

Limitations, reasons for caution: Baseline semen assessment was missing a bit more when cycles were successful, so that a bias connot be excluded. This weird result concerning semen parameters which appear to lower the live birth rate when they are good should thus be considered with caution.
Wider implications of the findings: Good baseline semen parameters do not appear as primordial for obtaining an IUI live birth, and were even found deleterious. However, thresholds highlighted in the present study were high, i.e. of limited clinical mean, and semen below them to remain normal. After confirming, explanations should be investigated: excessive acrosome reaction.
Trial registration number: Not applicable

P-128 Audit of testicular sperm in assisted conception for non-azoospermic infertile couples

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Study question: What live birth rate do we see when we use testicular sperm in ART for non-azoospermic couples after at least one previous failed cycle?
Summary answer: In our cohort of couples 24% had a live birth using testicular sperm and therefore was not higher than national average ART rates.
What is known already: There is increased interest in using testicular sperm in assisted reproduction technology (ART) to improve outcomes after previous failed cycles. Mehta et al. reported results of a 50% live birth rate using testicular sperm in the first cycle for couples with oligospermia and a history of failed cycles with ejaculated sperm. We aim to audit our results in a similar population of couples.
Study design, size, duration: St Peters Andrology Centre in London, United Kingdom completed 128 surgical testicular sperm retrievals reviewed between the two-year period of 2018-2019. We conducted a retrospective audit of their paper-based records to identify those couples with injectable sperm on their semen analysis and who had previous cycles attempts using ejaculated sperm.
Participants/materials, setting, methods: We identified 27 couples who underwent testicular sperm extraction despite having an ejaculated semen analysis with injectable sperm and at least one previous failed cycle. A systematic review of their paper and electronic medical record was conducted to assess live birth rates and fertilization rates from ART.
Main results and the role of chance: Couples had an average male age of 41 (range 31-60) and an average female age of 38 (range 30-45). The men had an average serum testosterone of 15 nmol/L (range 8-35 nmol/L) and an average serum FSH of 8.9 IU/L (range 1.7-30 IU/L). 59% (n=17) of men had a DNA fragmentation index completed with an average score of 41% (range 31%-51[Y1]%). In the women the mean serum anti-Müllerian hormone (AMH) was 15.8 pmol/l (range 1-64 pmol/l). With ejaculated sperm the fertilization rate was 59% (95% CI [27%, 59%]) and blastocyst conversion rate was 43% (95% CI [50%, 69%]). There was no statistical significance with testicular sperm where the fertilization rate was 58% (95% CI [51%, 65%]) and blastocyst conversion rate was 54% (95% CI [40%, 67%]). Overall, there were 7 clinical pregnancies in this population of couples. Of these clinical pregnancies, 2 miscarried and 5 progressed to a live birth. This audit yielded a live birth rate per cycle of 15% and a live birth rate per couple of 24%.
Limitations, reasons for caution: Limitations of the study are low number of patients and absence of a control group.

Wider implications of the findings: We recommend caution and further analysis going forward using testicular sperm in ART where ejaculated sperm in available.
Trial registration number: not applicable

POSTER VIEWING
EMBRYOLOGY

P-129 Follicular extracellular vesicles of women with polycystic ovarian syndrome inhibit oocyte maturation

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Study question: Does follicular extracellular vesicles of women with polycystic ovarian syndrome (PCOS-EVs) interfere with the quality of oocytes?
Summary answer: PCOS-EVs induced oxidative stress in the oocytes and inhibited oocyte maturation by increasing the abnormal mitochondria distribution and abnormal spindle rates.
What is known already: Polycystic ovarian syndrome (PCOS) is a common endocrine disorder in women of reproductive age, with a prevalence up to 10%. Women with PCOS are characterized by a clustering of features, including hyperandrogenism, polycystic ovarian morphology, and notably, anovulation. Although international guidelines recommend assisted reproduction techniques to be an effective resort for PCOS patients to conceive. However, even after overcoming ovulatory dysfunction via ovulation induction, the pregnancy outcomes of patients with PCOS were far from satisfying with lower fertilization, cleavage, and implantation rates, implicating that theoocyte quality of these patients are affected. Whereas the mechanisms have not been elucidated yet.
Study design, size, duration: Follicular fluid of PCOS patients (n=10) and healthy controls (n=10) were collected and used for extracellular vesicles (EVs) isolation via ultracentrifugation. Germinal vesicle (GV) oocytes collected from female ICR mice were cocultured with RIF-EVs or FER-EVs, respectively, and PBS served as a blank control. GV breakdown (GVBD) rate and maturation rate were calculated at two-hour and fourteen-hour of co-culture, respectively. Besides, oocyte mitochondria distribution, meiosis spindle morphology, and oxidative status were assessed in different groups.
Participants/materials, setting, methods: EVs were determined by western blotting, nanoparticle tracking analysis, and transmission electron microscopy. Fluorescence labeled EVs were used to visualize internalization by oocytes. Oocytes mitochondria and mitosis spindles were stained with fluorescence, and abnormal mitochondria rate or abnormal spindle rate was calculated. Reactive oxygen species (ROS) level was detected in the differently treated oocytes. Moreover, the expression of CAT, GSS, and SOD was determined in the oocytes using quantitative reverse transcription polymerase chain reaction.
Main results and the role of chance: Both PCOS-EVs and CTRL-EVs are bilayered vesicles, ranging from 100 to 150 nm, and enriched in Alix, TSG101, and CD9. EVs could be internalized by oocytes within one hour. After coculture, the GVBD rate was similar among the three groups; whereas the maturation rate was significantly decreased in the PCOS-EV group compared with CTRL-EV group or PBS group. In addition, the abnormal mitochondria distribution rate or abnormal spindle rate were significantly increased in the PCOS-EV group compared with PBS or CTRL-EV group. The ROS level was increased in the PCOS-EV group compared with CTRL-EV group, and the expression of CAT, GSS, and SOD was increased in the PCOS-EV-treated oocytes.
Limitations, reasons for caution: Our study did not identify the contents of PCOS-EVs and CTRL-EVs, and the molecular mechanisms of dysregulations induced by PCOS-EVs need further researches to investigate.
Wider implications of the findings: This work confirmed that EV-conducted cellular communication played an important role in oocyte development in women with PCOS. The dysregulation of oocytes induced by PCOS-EVs might be related to the poor oocyte quality of women with PCOS, which may provide a novel target to improve pregnancy outcomes of these patients.
Trial registration number: not applicable

P-130 The effect of time of ejaculation and time of processing of semen on fertilization and ongoing pregnancy in IVF/ICSI treatments.

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Study question: Does time between ejaculation and processing, and time between processing and insemination/injection affect fertilization rate (FR) and ongoing pregnancy rate (OPR) in IVF/ICSI treatments?
Summary answer: Increasing time between processing and insemination significantly decreased the OPR after IVF. FRs after IVF/ICSI and OPR after ICSI were not affected by different time-intervals.
What is known already: The choice for IVF or ICSI depends on semen quality, however, this doesn't affect the outcome of IVF/ICSI treatments (Mariappen et al 2018). After ejaculation, the percentage of motile spermatozoa decreases progressively at a rate of about 10%/hour (Makler 1979). According to the ESHRE-guideline, semen should be processed within 1 hour after ejaculation. In our laboratory, a validation was performed that confirmed a decrease in sperm motility after ejaculation. During incubation at 37°C after processing, the sample remained stable in incubation medium (unpublished data). Therefore, we analyzed the effect of handling time and incubation time with regard to IVF/ICSI outcomes.
Study design, size, duration: This retrospective data analysis examines the effect of time between ejaculation and processing using density-gradient centrifugation (handling time) and time between processing and insemination (IVF)/injection (ICSI) (incubation time) on the FR and OPR, irrespective of the initial semen quality. A total of 1488 oocyte pickups (844 IVF, 644 ICSI) were included from 1060 patients undergoing fertility treatment between 2017 and 2019. Oocyte pickups without oocytes, with oocyte vitrification, or with donor oocytes were excluded.
Participants/materials, setting, methods: Anonymized data were obtained from the laboratory database ProMISe. Handling time and incubation time of the semen incubated at 37°C and 5% CO2 were analyzed in relation to the occurrence of TFF (Total Fertilization Failure), FR and OPR. Linear and logistic regression was performed in SPSS version 25. In case of significant association, the data were adjusted for potential confounders, such as woman's age, semen quality before and after preparation, and number of oocytes.
Main results and the role of chance: This study shows that increasing the incubation time of the semen significantly reduced the OPR per ET in IVF treatments (from 30,8% within 3,5 hours to 24,1% after 6 hours) even after adjusting for the potential confouders. However, the OPR in ICSI treatments was not significantly affected by the incubation time (rather, there was an opposite trend). Also, the handling time of the semen did not significant effect the FR per OPU and the OPR per ET in IVF/ICSI treatments. The overall percentage of TFF was 3,5% and did not differ significantly between the IVF and ICSI treatments. Both handling time and incubation time did not have a significant effect on the occurrence of TFF. An explanation for the decrease in OPR in IVF treatments may be that increasing the incubation time at 37°C reduces the sperm quality as the capacitation reaction takes place too early, energy levels are reduced, DNA damage increases, or vacuoles arise in the sperm heads (Thijssen et al 2014, Jackson et al 2010, Peer et al 2007). Incubation at room temperature and reduction of the insemination time may improve OPR.
Limitations, reasons for caution: Retrospective study limitations (bias), no data on DNA fragmentation, incubation of semen only at 37°C after preparation.
Wider implications of the findings: Although it is recommended to produce semen at the IVF-department, our results show that an exception can be made, when production of a semen sample in a clinical setting is stressful, with no negative effect on the outcome. Furthermore, incubation-time at room temperature may have a positive effect on OPR.
Trial registration number: not applicable

P-131 Significance of the phenomenon of blastomere exclusion from compaction: Its relation to irregular cleavage, blastocyst development rate, and pregnancy rate

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Study question: When does blastomere exclusion from compaction increase and what effect does it have on the embryo?
Summary answer: More blastomere were excluded from compaction in embryos with irregular cleavage, resulting in lower blastocyst development rates, but no decrease in pregnancy rates at transfer.
What is known already: It has been reported that many of the chromosome analysis results of blastomere excluded from compaction were aneuploid, and pointed out that this exclusion may be related to the repair of blastocyst euploidy, but the effect of the number of excluded blastomere has not been reported.
Study design, size, duration: This is a retrospective study of 578 embryos that developed into morula with time-lapse monitoring by EmbryoScope (Vitrolife) in 2018-2019.
Participants/materials, setting, methods: The target embryos were classified into two groups: embryos with normal first and second cleavage (normal cleavage group) and embryos with irregular cleavage (dynamics of one cell dividing into three or more cells), called "direct cleavage", at either cleavage (DC group), and the number of blastomere excluded from compaction during morula formation was recorded and compared. The blastocyst development rate and single blastocyst transfer pregnancy rates of the two groups were compared.
Main results and the role of chance: There are 286 in the normal cleavage group and 292 in the DC group. The mean number of excluded blastomere was 0.76 and 3.55, respectively, which was significantly higher in the DC group (P<0.01). Good blastocyst (Gardner classification 4 or higher) development rate was 84.5% (239/283) and 65.8% (181/275), respectively, and high grade blastocyst (Gardner classification BB or higher) development rate was 43.9% (105/239) and 14.9% (27/181) of them, both significantly higher in the normal cleavage group (P<0.01). The single blastocyst transfer pregnancy rates were 31.6% (25/79) and 32.4% (11/34), and the miscarriage rates were 24.0% (6/25) and 27.3% (3/11), respectively, neither was there a significant difference between the two groups. So, direct cleavage increased the number of blastomere excluded from compaction, decreased the rate of morula to good blastocyst development and reduced blastocyst grade, but did not affect blastocyst transfer pregnancy rate and miscarriage rate.
Limitations, reasons for caution: Please note that all target embryos must have developed into morula or larger (embryos that did not develop into morula will not be included in the study).
Wider implications of the findings: Severe chromosomal aberrant blastomeres formed by direct cleavage were excluded from compaction, and the blastocyst development rate decreased due to a decrease in the amount of viable cells, but it is suggested that this blastomere exclusion mechanism is not related to euploidy after blastocyst development.
Trial registration number: not applicable

P-132 Centralized versus local embryologists scoring of blastocyst quality obtained in a large European multicenter clinical trial

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Study question: Does blastocyst quality scoring by central assessment deviate from local assessment and potentially lead to the selection of a different single blastocyst for transfer?
Summary answer: Central and local assessment provided the same quality classification (poor / good / top) in 69% of all blastocysts and 63% of all transferred blastocysts.
What is known already: Blastocyst quality is scored most frequently by three morphological parameters, namely expansion and hatching (EH) status, inner cell mass (ICM) grading and trophectoderm (TE) grading. The score is used to define the quality classification (poor / good / top) which determines which embryo is to be transferred or cryopreserved. Blastocyst scoring and grading can be highly subjective, which does influence the choice for transfer and cryopreservation. Time-lapse imaging technology captures additional input about embryo development as well as enables centralized data storage and sharing for independent central assessments.
Study design, size, duration: Pooled embryo analysis from a prospective, randomized, multicenter trial (RAINBOW) of 619 women undergoing ovarian stimulation with an individualized dose of follitropin delta in a long GnRH agonist protocol between May 2018 and January 2020. Blastocysts were centrally assessed using time-lapse images by two independent assessors and one adjudicator . Selection of the blastocyst for transfer by local assessment was based on morphological scoring and not on morphokinetic time-lapse parameters.
Participants/materials, setting, methods: Oocytes were fertilized by ICSI and cultured in the Embryoscopeâ (Vitrolife) up to day 5 for transfer or day 5/6 for cryopreservation. Embryos were assessed as either non-blastocyst or blastocyst. Blastocysts were graded centrally and locally at 116 hrs of development, based on EH status (1-6), ICM (A-D) and TE grading (A-D). Central assessors were blinded to local assessment and embryo transfer selection.
Main results and the role of chance: In total 4282 embryos were assessed centrally, of which 2046 day 5 embryos (48%) were adjudicated due to a scoring difference of at least one parameter between the two central assessors. In total 38% of day 5 embryos were judged as non-blastocysts and 62% as blastocysts of which 61% (i.e. 38% of all embryos) were determined to be of good or top quality.

Identical results in terms of quality classification (poor / good / top) were obtained for 69% of blastocysts between local and central assessment and in 78%, between the two central assessors. Moreover, central and local scoring were identical in 62% for EH status, 53% for ICM grading and 57% for TE grading.

For all transferred blastocysts (n=508), central and local quality assessment was aligned for 63%. The ongoing pregnancy rate following single blastocyst transfer (SBT) was 41% (202/489), and similar to when considering only the transfers for which the central assessment had the same or a higher classification than the local assessment (166/411=40%). In 16% of all SBT, central quality assessment gave a lower score for the transferred blastocyst than the central assessment. This discrepancy could potentially have led to transfer of a different blastocyst.
Limitations, reasons for caution: This trial included assessments made by embryologists from 20 IVF centres. Some centres has limited experience with time-lapse technology for morphological blastocyst scoring. Scoring could therefore have been affected by differences in focal planes, magnification and contrast compared to inverted microscopy, with potential influence on blastocyst scores and quality classification.
Wider implications of the findings: Local and central blastocyst quality classification based on morphology aligns well but remains subjective. Embryo assessment may benefit from using tools like artificial intelligence-based algorithms for a more objective analysis.
Trial registration number: NCT03564509

P-133 Monopronuclear (1PN) embryos can derive in healthy pregnancies

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Study question: Should 1PN embryos be considered suitable for transfer when normal development is observed at day 3 or day 5?
Summary answer: In IVF/ICSI cycles, 1PN zygotes are encountered in 2.7% of inseminated oocytes. Transfer of 1PN-embryos should be considered in the absence of suitable 2PN embryos.
What is known already: During in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) zygotes containing only a single pronucleus (monopronuclear, 1PN) are encountered in 1-7.7% of cases, while the display of two pronuclei is expected in a normally fertilised oocyte. A 1PN zygote can be of gynogenetic or androgenetic origin, but it can also be biparental. Gynogenetic and androgenetic 1PN embryos can be haploid or diploid, so a diploid 1PN embryo is not guaranteed to be normally fertilised. Generally, 1PN are discarded, as they have an increased risk for aneuploidy. However, sporadically they can develop into healthyl babies.

Study design, size, duration: 1PN-zygotes (n=1287, 2.7% from all inseminated oocytes) from 1-1-2016 up to 15-12-2020 were retrospectively evaluated. The development and fate (discarded/transferred/cryopreserved) of all embryos were recorded. Embryos were evaluated at day 2, 3 or 5 of development. The policy of our unit is that, in absence of 2PN embryos, normal developed 1PN-embryos can be transferred on day 3. Supernumerary 1PN embryos can be cryopreserved at blastocyst stage. Ongoing pregnancies from fresh embryo transfers (ET) were analysed.

Participants/materials, setting, methods: In 946 IVF/ICSI cycles, at least one 1PN zygote was observed (total 1287 embryos). ICSI with ejaculated, PESA or TESE sperm counted for a total of 795 embryos, IVF cycles for 494 embryos. Embryo evaluation was performed using a home-made numerical algorithm: A (top embryo; 150-200 points), B (regular embryo; 100-149 points) or C (poor embryo; 0-99 points). Monopronuclear embryos always scored lower than equal developed 2PN embryos. Blastocyst evaluation was according to Gardner score.

Main results and the role of chance: From the 795 ICSI embryos, 49 (6.1%) were used for fresh ET (26 scored quality A or B), and a total of 60 embryos developed to blastocyst and were cryopreserved. From these 49 ICSI transfers, 4 (8.1%) ongoing pregnancies were obtained, all 4 from DET (1PN+2PN embryo), from which one twin pregnancy was confirmed. From the 494 IVF embryos, 41 (8.3%) were used for fresh ET (24 scored A or B), and 62 blastocysts were cryopreserved. A total of 9/41 (22%) ongoing pregnancies were obtained: 5 from SET (1PN) and 4 from DET (1PN+ 2PN embryo). Therefore, in only five IVF cycles a confirmed pregnancy was observed from a 1PN embryo (all A-quality embryos).

Considering six ongoing pregnancies with complete certainty of monopronuclear origin from fresh tranfers could be confirmed from our retrospective data, we can conclude that although the live birth rate of these embryos is very low (around 0,5- 1.0%), they should not be discarded when development is normal and no dipronuclear embryos are present.

Limitations, reasons for caution: Cryo-thawing data is missing as these embryos were not differentially marked at freezing. Therefore, the cumulative pregnancies from monopronuclear embryos could be higher. Embryos were not evaluated in a time lapse system, so asynchronicity of PN formation could explain missing the right moment for evaluation, while normal fertilized.

Wider implications of the findings: Notably, IVF monopronuclear embryos display a higher developmental potential than those derived from ICSI. We suggest that, in absence of dipronuclear embryos, culture to blastocyst stage before considering fresh ET or cryopreservation will help differentiate viable 1PN embryos, reducing the higher chance of genetic anomalies and miscarriages.

Trial registration number: N.A.

P-134 Zygote morphokinetic parameters (ZMP) differs between fertilized and non-fertilized (in vitro maturation) oocytes

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Study question: Is there any difference on the ZMP between the fertilized and the non-fertilized oocytes in an IVM cohort?

Summary answer: The zona pellucida (zp_g) , cytoplasm greyscale（cm_g),the cytoplasm size (cm_size), radius (cm_r) and deviation (cm_d) showed different patterns from the two groups.

What is known already: We developed a convolutional neural network (CNN) based algorithm that provides instant and bias-free analytical outcomes of oocyte morphological segmentation. The mature but not-fertilized oocytes tend to be static while the fertilized oocytes are more dynamic for preparing its following biological events.

Study design, size, duration: This was a case-control study on oocytes including 631 normal fertilized oocytes and 100 IVM oocytes from 01/08/2017 to 31/12/2019 conducted in the Prince of Wales Hospital, The Chinese University of Hong Kong.

Participants/materials, setting, methods: We used the convolutional neural network (CNN) algorithm to segment the ZMPs of the cytoplasm and zona pellucida of the oocytes. The ZMPs include cm_g, cm_size, cm_r, cm_d, zp_g, thickness of zona pellucida and the area of perivitelline space. For the ZMPs that did not change with time, we used t-test to test the significance and for the parameters changed with time we used dynamic warp timing and similarity test to find the difference.

Main results and the role of chance: The IVM group had a higher intensity of zp_g of 142.03 (128.52-158.70) compared with the fertilized group of 137.04 (121.69-154.37). The cm_g of IVM group was higher than fertilized group [122.55 (114.87-137.62) vs 119.37(108.88-132.87)]. The cm_size, cm_r and cm_d as parameters changed with time and showed a different pattern in two groups. The IVM group decreased the cm_size faster than the fertilized group but the fertilized group had a more dynamic change in the shape of cytoplasm (cm_d) during the development. The cm_r changed with the same pattern of cm_size provided evidence supporting the finding above.

Limitations, reasons for caution: The ZMPs in the IVM group was captured for 21 hours from the first polar body extrusion. Though the duration was similar to the one from fertilized to the first cleavage. The morphology change during that period may not represent the holistic one of IVM oocytes.

Wider implications of the findings: The IVM oocytes have different morphokinetic performance from fertilized oocytes. We used a novel method based on CNN to confirm the differences between the two groups showing that our algorithm was able to describe the morphokinetic changes in a quantitative way and corresponded with embryologist's experience.

Trial registration number: The Hong Kong Obstetrical & Gynaecological Trust Fund

P-135 Artificial neural networks (ANNs) for live birth prediction in frozen embryo transfers: the strength of post-warmed blastocyst dynamics

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Study question: Does the post-warmed blastocyst dynamics have an impact over the likelihood of achieving a live birth?

Summary answer: Variables related to dynamics of vitrified/warmed blastocysts have shown a greater effect on the live birth prediction than only embryo morphological quality through artificial intelligence.

What is known already: Morphological dynamics of vitrified/warmed blastocysts were described by Coello et al., in 2017. The investigated markers were the thickness of zona pellucida (µm) and blastocysts area (µm2) after warming and before transfer, the area of the inner cell mass (µm2), time of initiation of reexpansion (in minutes), and presence of collapse or contraction. They found a correlation between blastocyst reexpansion and implantation rate and developed a hierarchical model for implantation prediction. In our study, we evaluated the post-warmed blastocyst dynamics for live birth prediction by using novel artificial intelligence techniques.

Study design, size, duration: This retrospective analysis included 415 vitrified/warmed blastocysts with known live birth data. Blastocysts after warming were placed in EmbryoScope (Vitrolife) immediately until embryo transfer. Embryo evaluation and selection were performed by senior embryologists according to fresh blastocyst morphology (before vitrification). Then, parameters related to post-warmed blastocyst dynamics were calculated. Finally, these variables and the embryo morphological grade before the vitrification were used as input data for ANNs optimized with genetic algorithm for live birth prediction.

Participants/materials, setting, methods: Blastocysts were vitrified and warmed by the Cryotop method (Kitazato,Biopharma). During the period between the warming procedure and the embryo transfer, the following variables were measured with the drawing tools provided by the EmbryoViewer workstation: zona pellucida thinning (µm), blastocyst expansion (um) and the speed of these two events (µm/h). Finally, multilayer perceptron neural networks were trained with data of 331 embryos by using the backpropagation learning algorithm and tested with data of 84 embryos.

Main results and the role of chance: We trained and tested three architectures of ANNs with different input variables as follows: post-warmed variables (thinning of the zona pellucida, blastocyst expansion, thinning speed and

expansion speed) and morphological grade (A, B or C) for ANN1, only post-warmed variables for ANN2 and only morphological grade for ANN3. The highest success rate when ANNs classified embryos as positive and negative live birth (LB+ and LB-) was achieved by combining post-warmed variables and morphological grade before embryo vitrification. The general accuracies for the blind tests were: 73.8% for ANN1, 66.7% for ANN2 and 71.4% for ANN3. Likewise, this combination achieved the highest AUC on test dataset to predict LB- (0.76 for ANN1, 0.74 for ANN2 and 0.67 for ANN3). However, the ANN2 trained with only post-warmed variables showed the best capacity to predict LB+ with an AUC of 0.73 (versus 0.46 for ANN1 and 0.5 for ANN3).
Limitations, reasons for caution: The main limitation is the subjectivity of manual annotations, although only one embryologist participated in this task.
Wider implications of the findings: The dynamics of vitrified/warmed blastocysts prior to embryo transfer could be more relevant variables than the morphological quality on day 5 before the cryopreservation. The analysis of embryo behavior after warming could improve clinical outcomes in frozen embryo transfers.
Trial registration number: none

P-136 Factors predicting clinical outcomes of 511 recipients of vitrified oocyte donation from an UK-regulated egg bank

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Study question: Do established donor and recipient clinical markers predict recipient clinical pregnancy and live birth rates (LBRs) in a vitrified oocyte donation programme?
Summary answer: Recipient BMI and previous miscarriages predicted cumulative LBR. Likelihood of clinical pregnancy and LBR was higher in recipients of donors aged 23-29 than donors 18-22.
What is known already: The influence of age on ovarian reserve underlies the upper limit of 35 years for UK donors. However, recent evidence suggests that oocyte aneuploidy rates follow an inverse U-shaped curve in relation to a woman's age. Conflicting evidence exists regarding the impact of other donor-related factors including BMI, AMH, oocyte yield and prior reproductive history on recipient outcomes. Moreover, the effect of recipient age, BMI, and reproductive history on oocyte donation outcome remains unclear.
Study design, size, duration: Retrospective cohort study of 325 altruistic oocyte donors matched to a total of 511 recipients. Only first donations taking place between January 2017 and December 2019 were included.
Participants/materials, setting, methods: All oocyte donors were altruistic volunteers aged 18-35 with no prior infertility diagnosis. Donor and recipient screening for suitability and safety was carried out according to the Human Fertilisation Embryology Authority guidelines. Backward stepwise logistic regression was used to identify donor, recipient and embryology parameters predictive of recipient primary outcomes defined as clinical pregnancy and live birth, either cumulative or after the first embryo transfer (ET).
Main results and the role of chance: A total of 705 fresh and frozen/thawed ETs were performed, of which 76% were elective single embryo transfers (eSETs) of blastocysts (96.5%), resulting in a cumulative clinical pregnancy and LBR of 83.5% and 70.5% respectively after 3 ETs. Recipient BMI and previous miscarriages were predictors of cumulative LBR ($p<0.05$). The ratio of transferrable embryos per oocytes received/fertilised and the number of ETs needed to achieve the intended primary outcome were predictors of cumulative clinical pregnancy and LBR ($p<0.05$). Donor age 18-22 was associated with lower incidence of recipient clinical pregnancy and live birth after the first ET, as compared to donor age 23-29 ($p<0.05$).
Limitations, reasons for caution: The present study included only healthy oocyte donors, thus conclusions may not apply to subfertile or less healthy women. Male factors were not accounted for.
Wider implications of the findings: We demonstrate the efficacy of vitrified oocyte donation treatment and identify recipient BMI, previous miscarriages and embryology parameters as predictors of cumulative LBR. Additionally, the choice of donors aged 18-22 instead of older donors is found not to be advantageous for increasing the chance of clinical pregnancy and live birth.
Trial registration number: not applicable

P-137 Male embryos take longer to develop to the blastocysts stage

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Study question: Are there any differences in developmental timings between male and female preimplantation embryos?
Summary answer: There is a tendency for statistical difference in the time to reach blastocyst stage for male embryos compared to female embryos
What is known already: Differences in gene expression and metabolic uptake between male and female preimplantation embryos have been found in animal models and humans. These differences could affect the developmental timings of embryos resulting in differences in either sex. Morphokinetic parameters can precisely assess developmental timings. Only a few studies have analyzed morphokinetic parameters between male and female preimplantation embryos and no consensus has been reached on whether there is any sex-specific difference. The objective of this study is to compare morphokinetic parameters between male and female preimplantation embryos to determine any sex-specific developmental differences.
Study design, size, duration: This is a retrospective study including 102 preimplantation embryos from February 2018 to February 2020. The morphokinetic parameters obtained from time-lapse records of each embryo were: time to pronuclear fading (tPNf), times to 2–8 cells (t2, t3, t4, t5, t6, t7, t8), time to start of blastulation (tSB) and time to full blastocyst stage (tB). A two-tailed Student's *t*-test was used to compare morphokinetic parameters between embryo sexes. A $p<0.05$ was considered statistically significant.
Participants/materials, setting, methods: The study included retrospective time-lapse data from preimplantation embryos giving rise to 51 baby boys and 51 baby girls, as seen at birth. This is a single-center study with standardized culture conditions. Embryos in both study groups issued from cycles with donated oocytes. Only elective blastocyst stage single-embryo transfers (SET) on day 5 were assessed.
Main results and the role of chance: A tendency to statistical difference (p=[0.1-0.05]) was observed for blastocyst-related morphokinetic parameters: tSB (mean time was 89.6±6.3 hours in male embryos vs. 86.9±8.1 hours in female embryos, p=0.06) and tB (100.2±5.9 hours versus 97.9±6.5 hours, p=0.07). Male embryos showed an increased average time of 2.7 hours to tSB and 2.3 hours to tB, while no differences were found in the mean times of all the other morphokinetic paraments measured ($p>0.50$): tPNf (~21.8±3.0 hours) t2 (~24.4±3.2 hours); t3 (~35.6±3.9 hours); t4 (~36.6±4.6 hours); t5 (~46.9±6.0 hours); t6 (~53.5±7.0 hours); t7 (~54.1±7.3 hours) and t8 (~54.1±7.3 hours). This finding suggests a sex-specific difference in reaching blastocyst stages.
Limitations, reasons for caution: The main limitation of the study is its retrospective nature and the small sample size. We analyzed the data of embryos leading to a live birth (high-quality embryos), therefore, caution should be made when generalizing results to non-implanting embryos (of potentially lower quality).
Wider implications of the findings: Sex-specific differences in developmental timings of preimplantation embryos at blastocyst stage, as evidenced by time-lapse data, should be considered to avoid selection biases during embryo transfers in ART clinic.
Trial registration number: not applicable

P-138 When is low quality really low? Should we transfer low-grade blastocysts?

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Study question: What is the live birth rate after single, low-grade blastocyst (LGB) transfer?
Summary answer: The live birth rate for LGBs is 28%, ranging between 15-31% for the different inner cell mass (ICM) and trophectoderm (TE) subgroups of LGBs.
What is known already: Live birth rates following LGB transfer are varied and have been reported to be in the range of 5-39%. However, these estimates are inaccurate as studies investigating live birth rates following LGB transfer are inherently limited by sample size (n=10-440 for LGB transfers) due to LGBs being ranked last for transfer. Further, these studies are heterogenous with varied LGB definitions and design. Collating LGB live birth data from multiple clinics is warranted to obtain sufficient numbers of LGB transfers to establish reliable live birth rates, and to allow for delineation of different LGB subgroups, including blastocyst age and female age.
Study design, size, duration: We performed a multicentre, multinational retrospective cohort study in 9 IVF centres in China and New Zealand from 2012 to 2019. We studied the outcome of 6966 single blastocyst transfer cycles on days 5-7 (fresh and frozen) according to blastocyst grade, including 875 transfers from LGBs (<3bb, this being the threshold typically applied to LGB studies). Blastocysts with expansion stage 1 or 2 (early blastocysts) were excluded.
Participants/materials, setting, methods: The main outcome measured was live birth rate. Blastocysts were grouped according to quality grade: good-grade blastocysts (GGBs; n=3849, aa, ab and ba), moderate-grade blastocysts (MGBs; n=2242, bb) and LGBs (n=875, ac, ca, bc, cb and cc) and live birth rates compared using the Pearson Chi-squared test. A logistic regression analysis explored the relationship between blastocyst grade and live birth after adjustment for the confounders: clinic, female age, expansion stage, and blastocyst age.
Main results and the role of chance: The live birth rates for GGBs, MGBs and LGBs were 45%, 36% and 28% respectively (p<0.0001). Within the LGB group, the highest live birth rates were for grade c TE (30%) and the lowest were for grade c ICM (19%). The lowest combined grade (cc) maintained a 15% live birth rate (n=7/48). After accounting for confounding factors, including female age and blastocyst characteristics, the odds of live birth were 2.33 (95% CI = 1.88-2.89) for GGBs compared to LGBs and 1.56 (95% CI = 1.28-1.92) for MGBs compared to LGBs following fresh and frozen blastocyst transfers (p<0.0001, odds ratios confirmed in exclusively frozen blastocyst transfer cycles). When stratified by individual ICM and TE grade, the odds of live birth according to ICM grade were 1.31 (a versus b; 95% CI = 1.15-1.48), 2.82 (a versus c; 95% CI = 1.91-4.18) and 2.16 (b versus c; 95% CI = 1.48-3.16; all p<0.0001). The odds of live birth according to TE grade were 1.33 (a versus b; 95% CI = 1.17-1.50, p<0.0001), 1.85 (a versus c; 95% CI = 1.45-2.34, p<0.0001) and 1.39 (b versus c; 95% CI = 1.12-1.73, p=0.0024).
Limitations, reasons for caution: Despite the large multicentre design of the study, analyses of transfers occurring within the smallest subsets of the LGB group were limited by sample size. The study was not randomised and had a retrospective character.
Wider implications of the findings: LGBs maintain satisfactory live birth rates (averaging 28%) in the general IVF population. Even those in the lowest grading tier maintain modest live birth rates (15%; cc). It is recommended that LGBs not be universally discarded, and instead considered for subsequent frozen embryo transfer to maximize cumulative live birth rates.
Trial registration number: Not applicable

P-139 The role of hormones and LH receptor expression of granulosa cells collected from large and small follicles in natural/minimal stimulation cycle IVF

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Study question: Do different follicle sizes influence gonadotropins (LH, FSH) and sex steroid (estradiol) in follicular fluids and LH receptor expression (LHCGR) in cumulus oocyte complexes (COCs)?
Summary answer: It was found that differences in levels of FSH, estradiol values and LHCGR mRNA expression level in COCs between small and large follicles.
What is known already: The maturity rate in oocytes of small follicle is significantly lower compared to that of large follicles.
Study design, size, duration: After obtaining written consents from 78 infertile patients, we aspirated the large (>15 mm) and small (<5 mm) follicles, and collected follicular fluids at oocyte retrieval.
Participants/materials, setting, methods: We measured levels of LH, FSH and estradiol by enzyme immunoassay from large and small follicular fluids after oocytes retrievals. All collected oocytes were distinguished from large and small follicles, we confirmed the maturity of retrieved oocytes by the presence of first polar body. Then we extracted total RNA from granulosa cells and measured mRNA expression of *LHCGR*, encoding the human LH receptor, by quantitative real-time PCR. Each value was normalized to *ACTB* mRNA levels.
Main results and the role of chance: LH levels were nearly equal between small and large follicles (P=0.8356). Whereas FSH and estradiol levels were significantly lower in small follicles (P<0.0001). The expression levels of *LHCGR* mRNA were significantly lower in small follicles than in large follicles during natural cycles. The maturity rate in oocytes of small follicle was significantly lower compared to that of large follicles (96.0% vs. 21.7%, P<0001).
Limitations, reasons for caution: The main limitation of the present study was collected by 42 natural cycles and 36 mild stimulation cycles with letrozole following low-dose clomiphene.
Wider implications of the findings: In spite of almost the same LH levels between two groups, the reason why the significantly lower maturation rates of oocytes collected from small follicles is poor LHCGR mRNA expression due to insufficient granulosa cells glowth because of low FSH and estradiol levels.
Trial registration number: not applicable

P-140 Zygote morphokinetics as a predictor of blastocyst quality

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Study question: Does the total cytoplasmic volume (TCV) of the zygote and time of pronuclei disappearance (tPNf) affect blastocyst development potential and blastocyst quality?
Summary answer: The total cytoplasmic volume of zygote and the time of pronuclei disappearance strongly affect blastocyst development as well as blastocyst quality.
What is known already: Extended embryo culture with blastocyst transfer is considered a useful method for selecting embryos with a high implantation potential. One of the main concerns in the German Embryo Protection Act is that the choice of embryos that have a high potential for successful implantation must be made at the pronucleus stage. The introduction of time-lapse technology enabled comprehensive information regarding the morphology and kinetics of the embryo. Although for the past years, strong correlations between embryo morphokinetics and positive outcomes have been demonstrated, some disagreement concerning the wide application of these parameters into an early-stage embryo selection is still present.

Study design, size, duration: The injection time of ICSI was designated as "time zero", and computer software was used to calculate the time frame between the injection and the moment of pronuclei disappearance (tPNf). The total cytoplasmic volume was calculated 16–18 h after injection based on manually drawn diameters of the zygotes, by the computer. Obtained measurements were later associated with the blastocyst formation potential as well as with blastocyst quality.
Participants/materials, setting, methods: A total of 187 oocytes from 34 patients undergoing the antagonist cycle for ICSI treatment were evaluated. All blastocysts were cultured in Embryoscope™ according to the manufacturer's specifications (Vitrolife, Sweden). The Gardner and Schoolcraft scoring system was used to describe blastocyst quality. Statistical analyses were performed using IBM SPSS version 24. Data were reported as median and range. Differences between groups were tested using the Mann-Whitney U test. Statistical significance was defined as $p<0.05$.
Main results and the role of chance: Obtained data showed that the total cytoplasmic volume values were significantly higher in zygotes that reached the blastocyst stage compared with those that did not [708376,268μm³ (560564,412μm³ - 838602,605μm³) vs 674349,917 (415749,353- 823640,638) respectively; $p<0.0001$]. Furthermore, this parameter as well significantly affect blastocyst quality where zygotes which formed better blastocyst quality had smaller cytoplasmic volume [685568,079 μm³ (560564,412- 790112,397μm³) vs 745514,662 μm³ (616581,339- 838602,605) respectively; $p<0.0001$]. Time of pronuclei disappearance (tPNf) was significantly different ($p<0.0001$) in successfully formed blastocysts [22.00h (17-29 h)] versus arrested or non-blastulating embryos [23.00h (17-56 h)]. Zygotes who had a shorter time frame between injection and pronuclei disappearance showed better blastocyst quality values compared with those who had longer time frame [21.00h (17-28h) vs 23.00h (18-29h) respectively; $p<0.01$].
Limitations, reasons for caution: The limitation of the presented study was that due to the double-embryo transfer correlation between morphokinetic parameters and pregnancy rate was not possible to be calculated. Further research should link these morphokinetic parameters with pregnancy rate and live birth rate as well.
Wider implications of the findings: The potential of the present findings is considerable, especially for countries with strict Embryo Law Regulation. Obtained results might be highly useful for selecting embryos with high implantation potential. In addition, the present work illustrates the possibility of additional information that can potentially be incorporated into an embryo classification model.
Trial registration number: not applicable

P-141 Artificial intelligence system for the automation of the blastocyst morphology evaluation in GERI Time-lapse Incubator

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Study question: *Can an Artificial Intelligence (AI) system (hand-crafted vs. deep learning techniques) based on single embryo image analysis from a GERI time-lapse incubator (TL) evaluate the blastocyst morphology?*
Summary answer: Our hand-crafted method trained with blastocyst images from Geri-TL evaluated and classified parameters regarding to embryo quality with a global precision of 63.7% in blind-test.
What is known already: Recent studies have shown that AI can improve automatic grading and embryo selection. The approaches that have been carried out are very different, but all they conclude that there is a great potential (Rad2019, Manoj2020, Thirumalaraju2020). As we know, conventional embryo evaluation is performed manually based on the morphology of the blastocyst, therefore, it should be possible to replicate this process. In this study, we implemented different methods to analyse the behaviour and performance of an AI doing embryology tasks.
Study design, size, duration: Our study consisted of a retrospective analysis for the automatization of embryo evaluation with different approaches. We developed our models based on 715 images extracted from GERI TL Videos (Genea, Australia) from a single IVF center. Database was divided into 3 classes depending on the quality of the embryo according to ASEBIR morphology criteria (high; medium and low-quality). All the images were divided into 70% for training, 15% for validating and 15% for testing.
Participants/materials, setting, methods: We developed an automated AI algorithm to extract and classify features from images at 111,5 hpi of embryos cultured in GERI TL. Hand-crafted features from texture information are extracted to feed the classification algorithm. A statistical analysis is carried out to select the more discriminative variables. Parallelly, a deep neural network was built to compare performance of automatic and hand-crafted features. Additionally, we trained a model to detect embryo in the well.
Main results and the role of chance: High-quality, medium-quality and low-quality sensitivity were 73%, 56% and 72% for hand-crafted method and 76%, 53% and 22% for deep learning approach, respectively. High-quality, medium-quality and low-quality precision were 66%, 56% and 76% for hand-crafted method and 40%, 60% and 55% for deep learning approach, respectively. The global accuracy associated with each method was 64% and 50%. Also, we noticed that results were higher when we applied our embryo masks that avoid irrelevant information. In this initial attempt, our results showed that it is possible to replicate the embryo evaluation process.
Limitations, reasons for caution: The low results obtained in our deep learning model due to the absence of an extent dataset did not allow to obtain a model applicable to the clinic. However, the preliminary study let us to conclude the high potential of the approach.
Wider implications of the findings: Our results showed a potential automatization of the embryo evaluation process in Geri TL where the available software for embryo selection does not provide such option. Our findings leaded to an increase in objectification, a reduction of the workload of the embryologist and the research of new unknown morphological variables.
Trial registration number: not applicable

P-142 Comparison of the IVF outcome in the course of ICSI vs PICSI treatment continuation in exactly the same group of HBA abnormal patients

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Study question: Does the PICSI have a beneficial effect for men with abnormal HBA on the fertilization rate, blastocysts number and clinical pregnancies in the next attempt?
Summary answer: Patients with HBA <80% choosing to undergo PICSI after ICSI failure see an increase in blastocyst and pregnancy rates.
What is known already: Hyaluronic acid (HA) is a main component of cervical mucus and the extracellular matrix of cumulus cells. The formation of HA-binding sites in sperm cell membranes is one of the markers of sperm maturation indicating completion the spermatogenic process of remodelling the plasmatic membrane, cytoplasmic extrusion and nuclear maturity. Spermatozoa selected by the HA-binding technique (the physiologically selected intracytoplasmic sperm injection – PICSI) have a potentially reduced risk of chromosomal aneuploidy or DNA fragmentation. Recent evidence do not show significant benefits in using PICSI. However, it has not been analysed in the course of treatment continuation in the same patients.
Study design, size, duration: This was a retrospective case-control study. It included exactly the same 58 patients with abnormal HBA, who underwent IVF treatment with ICSI initially and later with PICSI, between January 2014 and October 2020 at INVICTA Fertility Centre, Poland. Median female partner age in PICSI group was 36,2±5,34, without PICSI 35,8 ±5,28.
Participants/materials, setting, methods: 275 cycles (130 ICSI and 145 PICSI) resulted in 793 and 897 MII respectively. Patients were also divided into two groups <80 % and ≥80% depending on the obtained HBA score expressed as the percentage of sperm bound with hyaluronan. The analysis covered the

fertilization rate (FR), TQ and total blastocyst rate on day 5 and clinical pregnancy rate. Patients with poor response to stimulation were excluded from the study.
Main results and the role of chance: FR in ICSI and PICSI groups was not significantly difference (57.00%±31.2 vs 59.87%±30.8) even when taking into account the division of patients according to the obtained HBA score. In the <80% group the FR was 57.04%±29.3 vs 59.54%±30.8 in ICSI vs PICSI group respectively.

There were no significant differences when comparing the under HBA ≥80% subgroups for all analysed outcomes. Fertilization rate was 56.88% in the ICSI group vs 61.03% in the PICSI group. The percentage of blastocysts was 28.61% vs 34.45% and the percentage of TQ blastocysts on day 5 was 15.32% vs 16.81% with ICSI and PICSI respectively, in the group consisting of the same patients.

In the HBA <80% group significant differences were observed in the percentage of obtained blastocysts 37.81% vs 47.61% by comparing the ICSI and PICSI approaches (p<0.05). Also, percentage of TQ blastocyst on day 5 also was higher in patients with <80% HBA score after PICSI and was statistically significant (17.07% ICSI vs 23.92% PICSI, p<0.05). We saw statistically significant (p<0.01) increase in percentage of clinical pregnancies from 29.03% without PICSI to 69.44% in patient's subsequent procedures involving PICSI.
Limitations, reasons for caution: More data is required to confirm that improved results of PICSI procedure are consistent and possible to reproduce in a larger group – and as a result could be included as part of the standard treatment process.
Wider implications of the findings: The presented results show that in patients with normal HBA score, PICSI does not bring a measurable benefit and this may be important factor to consider in decision-making for couples seeking assistance.
Trial registration number: not applicable

P-143 Improved embryonic development and utilization rates with EmbryoScope: A within-subject comparison versus a benchtop incubator

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Study question: In consecutive intracytoplasmic sperm injection (ICSI) cycles, is embryonic development in EmbryoScope better than the previous one obtained in a benchtop (G-185) incubator?
Summary answer: Embryonic development, and oocyte and embryo utilization rates (OUR and EUR) are significantly improved in the EmbryoScope, as compared to G-185.
What is known already: The time-lapse imaging (TLI) system, which allows a non-invasive continuous assessment of embryo morphokinetics parameters in a closed culture system has been developed, promising improved embryo development by reducing oscillations in pH, humidity and temperature. To investigate this hypothesis, one study has already compared embryonic development in a TLI versus a benchtop incubator. However, It has never been investigated whether embryonic development can be improved within-subject, by changing from benchtop incubator in the first intracytoplasmic sperm injection (ICSI) cycle to the EmbryoScope, a TLI incubator, in the following ICSI cycle, and that was the objective of the present study.
Study design, size, duration: This study had a retrospective within-subject design, in which each cycle served as its own control. Data were obtained via chart review of patients undergoing ICSI in a private university–affiliated IVF center that fulfilled the following criteria: second ICSI attempt in which embryos had been cultured in a TLI incubator system (TLI group, n=71), preceded by a first ICSI attempt in which embryos had been cultured in a conventional incubator (Control group, n=71).
Participants/materials, setting, methods: Embryonic development up to the fifth day of development, OUR (transferred embryos plus frozen embryos per retrieved oocytes) and EUR (transferred embryos plus frozen embryos per fertilized oocytes) were compared between the groups using generalized linear models followed by Bonferroni post hoc. The post hoc achieved power was 82.6%, considering the sample size, the effect size obtained for blastocyst development rate and 5% significance level.
Main results and the role of chance: There were significant differences in fertilization rate (76.0% ± 1.3 vs. 80.0% ± 1.4, p=0.044, OR: 1.051, CI: 1.001 – 1.103), non-fertilization rate (14.8% ± 0.6 vs. 6.3% ± 0.4, p<0.001, OR: 0.424, CI: 0.370 – 0.486), day-2 non-cleavage rate (3.8% ± 0.2 vs. 1.1% ± 0.1, p<0.001, OR: 0.285, CI: 0.234 – 0.347), blastocyst development rate (40.9% ± 1.1 vs. 55.6% ± 1.3, p<0.001, OR: 1.358, CI: 1.267 – 1.456), frozen blastocyst rate (31.8% ± 0.8 vs. 37.0% ± 0.9, p<0.001, OR: 1.163, CI: 1. 085 – 1.248), OUR (40.7% ± 1.0 vs. 50.2% ± 1.1, p<0.001, OR: 1.232, CI: 1.155 – 1.314), and EUR (52.4% ± 1.1 vs. 66.6% ± 1.2, p<0.001, OR: 1.269, CI: 1.202 - 1.341), all in favor of TLI group. Pregnancy rate (30.2% vs. 30.8%, p=0.940), implantation rate (24.6% ± 40.0 vs. 26.1% ± 41.6, p=0.830), and miscarriage rate (21.1% vs. 15.0%, p=0.622) were similar between Control and TLI groups, respectively.
Limitations, reasons for caution: (i) Different culture dishes were used in each system; (ii) it is not possible to confirm how much of the embryonic improvement was due to the culture conditions; (iii) the study design is not ideal for the comparison of clinical outcomes and, also, underpowered to do so.
Wider implications of the findings: Even though the clinical outcomes were similar between the groups, the results may also lead to higher cumulative pregnancy outcomes following embryo thawing and transfer.
Trial registration number: Not applicable

P-144 Undisturbed culture in time-lapse systems improves embryo development and quality

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Study question: Does culture in integrated time-lapse systems (TLS) improve embryo development and blastocyst quality compared to conventional benchtop incubators (CI), within the same IVF laboratory?
Summary answer: Under similar conditions, culture in TLS resulted in a significant increase in blastocyst rate, top quality blastocyst rate and proportion of biopsied embryos per treatment
What is known already: Integrated TLS have the potential of delivering a stable and undisturbed environment throughout the whole embryo culture, avoiding taking them out for assessment. However, there is still lack of quality evidence of the performance of these incubators compared to CI at supporting embryo culture until blastocyst stage. Studies abording this issue are still scarce, heterogeneous and have a small sample size. Although some authors have reported an improvement in embryo development and quality using TLS, global results are inconsistent. To our knowledge, the present study evaluates the effect of TLS on embryo quality on the largest sample size yet.
Study design, size, duration: Unicentric retrospective cohort study including 14248 ICSI treatments from 2016 to October 2020, with both autologous and donated oocytes. We compared blastocyst rate (BR) and proportion of top-quality blastocysts (TQB=Morphology ASEBIR score A) per treatment between those using TLS (N=7500) and CI (N=6748), and the proportion of embryos biopsied (EB) in cycles with pre-implantation genetic testing (PGT-A; N=2642). We performed a sub-analysis in treatments using single-step culture medium (N-TLS=4398, N-CI=1140) in both types of incubators.
Participants/materials, setting, methods: Embryo cohorts were cultured until blastocyst stage in one of 3 TLS: EmbryoScope, EmbryoScope Plus (Vitrolife,) and Geri (Genea Biomedx), or in a CI (ASTEC). Embryo quality was assessed following ASEBIR morphological criteria. Culture protocols and media changed during the included time period. For that reason, we did a sub-study in the treatments performed since the implementation of Gems® (Genea Biomedx) single-step (SS) culture medium in all incubators. Statistical analysis was done using ANOVA tests.
Main results and the role of chance: Treatments were differently distributed and heterogeneous in terms of number of oocytes obtained per patient, so we stratified the analysis according to ovum origin and compared mean rates per cycle instead of total number of embryos per group. BR was statistically higher (*P*<0,001) in the TLS group, in both autologous (62,98±29,37% vs 59,49±31,09%

in CI) and oocyte donation treatments (69,25±22,07% vs 66,27±23,28% in CI). Proportion of TQB was also significantly higher in the TLS in both types of cycles (*P*<0,001): 3,60±12,29% in TLS vs 2,27±9,71% in CI in autologous cycles, 8,68±15,31% in TLS vs 7,32±14,02% CI in ovum donation cycles. Results were corroborated in the SS media sub-study (*P*<0,05): BR was 63,87±29,23% in TLS vs 57,53±30,61% in CI with autologous oocytes, and 70,76±21,63% in TLS vs 67,39±22,68% in CI with donated oocytes; TQB rates were 3,66±12,06% in TLS vs 2,05±9,26% in CI in autologous treatments and 8,81±15,21% in TLS vs 6,84±12,91% in CI in ovum donation treatments. Regarding PGT-A treatments, we found no significant difference in the biopsy rate in the total comparison, although the rate significantly increased in the TLS group since the implementation of single-step medium (52,36±24,69% in TLS vs 48,63±22,56% in CI; *P*=0,007)

Limitations, reasons for caution: Not only culture conditions varied over time, but also the number of TLS in the laboratory, which increased lately. Hence, even though the most recent treatments included in the all-SS sub-study are more homogeneous in terms of culture conditions, they are unbalanced regarding the distribution among incubators.

Wider implications of the findings: Our results demonstrate the superiority of TLS coupled with single-step culture media against traditional embryo culture systems at supporting embryo development. The optimal environment provided by TLS enhances embryo development until blastocyst stage as well as their quality, increasing the cumulative chances of getting a life-birth for each patien.

Trial registration number: not applicable

P-145 usefulness of morphokinetic data to predict pregnancy rates of day-6 blastocyst transfers

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Study question: Can a scoring model based on morphokinetic data developed to predict pregnancy rates of day-5 blastocyst transfers (KIDSCORE™D5) predict pregnancy rates of day-6 blastocyst transfers?

Summary answer: KIDSCORE™□D5 was able to predict the clinical pregnancy rates of embryo transfers done on day 6 with an area under the curve (AUC) of 0.72.

What is known already: KIDSCORE™□D5 is a scoring model based on morphokinetic data developed to predict the pregnancy rates of day-5 blastocysts. In 2019, Regnier et al. reported that the AUC of KIDSCORE™□D5 for predicting clinical pregnancy rates of day-5 blastocyst transfers was 0.6. However, as KIDSCORE™□D5 is constructed based on morphological characteristics and developmental dynamics of day-5 blastocysts, it is unclear whether KIDSCORE™□D5 can predict pregnancy rates of day-6 blastocyst transfers. Since there are many cases of day-6 blastocyst transfers, it is important to know if KIDSCORE™□D5 can predict pregnancy rates of day-6 blastocyst transfers.

Study design, size, duration: This retrospective single-center study, which included 162 day-5 and 72 day-6 blastocyst transfers, respectively, was conducted at Takahashi Women's clinic from January to December 2019. Blastocysts derived from 146 patients who underwent intracytoplasmic sperm injection. All blastocysts were cryopreserved and were transferred singly.

Participants/materials, setting, methods: We used EmbryoScope+™□ (Vitrolife) for *in-vitro* culture and calculated KIDSCORE™□D5 (ver.3) using Embryoviewer™□ (Vitrolife). Blastocyst scoring was done from 1.0 to 9.9. Clinical pregnancy was defined as the presence of a gestational sac confirmed by transvaginal ultrasonography. Statistical analysis was performed with JMP Pro 15.00 (SAS). The relationship between KIDSCORE™□D5 and clinical pregnancy was evaluated by the AUC using ROC curve analysis and multivariate analysis adjusted for patient age.

Main results and the role of chance: The mean KIDSCORE™□D5 of day-5 and day-6 blastocysts was 7.1±1.7 and 3.7±1.5, respectively. KIDSCORE™□D5 of day-6 blastocysts was significantly lower than that of day-5 blastocysts (p< 0.0001, Wilcoxon test). ROC curve analysis showed that the KIDSCORE™□D5 could predict clinical pregnancy rates with an AUC of 0.62 for day-5 blastocysts and 0.72 for day-6 blastocysts. The cut-off values for KIDSCORE™□D5 were 5.7 and 4.9 for day-5 and day-6 blastocysts, respectively. Blastocysts above the cut off value on both day-5 and day-6 had a significantly higher pregnancy rate than those below the cut off value (day-5: 61.9% vs. 33.3%(p=0.0023), day-6: 47.4% vs. 7.6%(p=0.0003)). Multivariate analysis adjusted for patient age showed that KIDSCORE™□D5 correlated with clinical pregnancy rates of days 5 and 6 of blastocyst transfer with AUCs of 0.66 and 0.73, respectively.

Limitations, reasons for caution: This study had a small sample size, and it was a retrospective single-center study. In addition, the relationship between KIDSCORE™□D5 and clinical pregnancy rates may vary among facilities. Therefore, a prospective multicenter validation is necessary.

Wider implications of the findings: Our study results indicated that KIDSCORE™□D5 predicted clinical pregnancy and that morphokinetic parameters related to clinical pregnancy were similar between day-5 and day-6 blastocysts. Hence, morphokinetic evaluation can serve as a criterion for deciding which of multiple day-6 blastocysts can be transferred.

Trial registration number: Not applicable

P-146 Differential impact of three embryo culture media for IVF on in vitro development and perinatal outcome: a single-center RCT

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Study question: This report provides updated data from an RCT determining which embryo culture medium yields optimal IVF outcomes.

Summary answer: Embryo culture systems used for IVF differentially affected preimplantation development and resultant obstetric and perinatal outcomes, including birthweights of live-born singletons.

What is known already: Currently, multiple embryo culture medium systems are in use for IVF, raising questions regarding which is optimal. However, the ability of a medium to yield preimplantation embryos is not necessarily indicative of embryo viability. For example, supplementation of medium with serum was commonly used to increase animal blastocyst yields, but this impaired embryonic, fetal, and offspring health. In humans, medium composition and culture duration can influence IVF efficacy and offspring phenotype. Given the importance of culture systems in determining clinical outcomes, existing data regarding differential culture system impacts are insufficient and additional well-designed studies are required.

Study design, size, duration: Between February 2016 and August 2017, 795 couples undergoing their first autologous clinical IVF cycle and freeze-all strategy were recruited. Participants were randomized via computer-generated tables into three groups. Following standard oocyte retrieval and IVF/ICSI procedures, embryos were cultured using three different culture media, G1 Plus/G2 Plus (G1/G2; Vitrolife), Global Total (GT; LifeGlobal), or Sequential Cleav/Sequential Blast (SC/SB; Origio). Thirty-eight patients exhibiting no 2PN oocytes following insemination or those undergoing fresh embryo transfers were excluded.

Participants/materials, setting, methods: For patients yielding a single good-quality cleavage-stage (day-2 or day-3) embryo, that cleavage-stage embryo was vitrified. For patients yielding two or more good-quality cleavage-stage embryos, two or less good-quality cleavage-stage embryos were vitrified. The culture period of the remaining embryos was extended, and all good-quality blastocyst-stage (day-5 or day-6) embryos were vitrified. This report presents data for vitrified embryo transfer performed until the end of December 2020.

Main results and the role of chance: The mean per-cycle vitrified embryo yield (± SD) was comparable between groups for cleavage-stage embryos, but significantly different for blastocyst-stage embryos (G1/G2: 1.69 ± 2.2, GT: 2.53 ± 3.01, SC/SB: 2.04 ± 2.42; P = 0.001). Following vitrified cleavage- or blastocyst-stage embryo transfers, biochemical pregnancy rates were significantly different between groups (G1/G2: 55.6 %, GT: 59.1 %, SC/SB: 46.2 %; P = 0.011). Furthermore, a between-group trend towards different live birth rates was observed (G1/G2: 41.7 %, GT: 42.1 %, SC/SB: 33.1 %; P = 0.063). Of 382 live births, data for first-borns (n = 323; 295 singletons and 14 twin-pairs) are reported here. Perinatal data did not differ significantly between groups for both cleavage- and blastocyst-stage embryo transfers, including gestational age- and gender-adjusted singleton birthweight (z-score). Following multiple linear regression (including selected covariates), adjusted mean singleton birthweights were

significantly lower in the G1/G2 and GT groups than in the SC/SB group (by 131 g; P = 0.011 and 110 g; P = 0.032, respectively) and tended to be lower for cleavage-stage embryo transfers than for blastocyst-stage embryo transfers (by 102 g; P = 0.053).

Limitations, reasons for caution: A larger cohort size and longer-term follow-up are required to verify and further elucidate the impact of embryo culture methods on child health. Such studies will raise awareness regarding the sensitivity of *in vitro*-cultured human embryos to their environment, ultimately resulting in practices that decrease IVF risks to offspring.

Wider implications of the findings: Pregnancy outcome of the medium yielding fewer blastocysts was comparable or superior to that of other media, highlighting the importance of differentiating between the ability to support pre-implantation development versus the ability to yield viable embryos. Embryo culture medium had a greater impact than embryo transfer stage on live birthweight.

Trial registration number: UMIN000020910

P-147 The impact of blastocyst morphological parameters on live birth and singleton birthweight in single blastocyst transfer cycles

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Study question: Does blastocyst morphological parameters: blastocyst expansion degree(Expansion), inner cell mass (ICM), and trophectoderm (TE) grades affect live birth and singleton birthweight in single blastocyst transfer cyles?

Summary answer: The effects of blastocyst morphological parameters on live birth and singleton birthweight are different between biopsied blastocysts cycles and non-biopsied blastocysts cycles.

What is known already: It has been known that blastocysts with highest scores for three blastocyst morphological parameters achieve highest pregnancy rates, however, very few studies have comparatively analysed the effect of individual parameters on live birth and singleton birthweight about single blastocyst transfer cycles.

Study design, size, duration: This retrospective study involved all single blastocyst transfers cycles and their live birth outcome and singleton birthweight during the period from January 2014 to August 2019 at a tertiary care center.

Participants/materials, setting, methods: A total of 28515 single blastocyst transfer cycles were available for analysis and were divided into four groups: biopsied blastocysts cycles (BBC), thawed blastocysts cycles(TBC), blastocysts from thawed cleavage embryos cycles(BTCEC) and fresh blastocysts cycles(FBC). The primary outcome were live birth and singleton birthweight. Multiple logistics regression and linear regression analyses were respectively performed to investigate the effect of blastocyst morphological parameters on live birth and birthweight after adjusting potential confounders.

Main results and the role of chance: While analyzing the effect on live birth, we found that live birth of grade B ICM were lower than grade A ICM , live birth of grade C TE were lower than grade A TE and Expansion doesn't matter. Those result were same in three kinds of non-biopsied cycles. While all three parameters were statistically independently significant in biopsied blastocysts cycles.

While analyzing the effect on singleton birthweight, only Expansion was found to be statistically significant in biopsied blastocysts cycles, and birthweight of Expansion grade 5 was lower than Expansion grade 6(P=0.005),with a mean difference of 57g(3375.12±527.91 versus 3318.42±510.33). Limitations, reasons for caution: Most blastocysts with poor grade, especially ICM grade C, were not transferred, then the effect of poor grade such as ICM grade C were still unknown.

Wider implications of the findings: The study identified the association between blastocyst morphological parameters and live rate and compared the relative importance of three parameters in different kind of cycles through large size comparative analysis, which would help selecting high-quality embryos for transfer.

Trial registration number: not applicable

P-148 Effect of additional laser assisted drilling (LAD) during trophectoderm (TE) biopsy on mosaicism rate

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Study question: Does applying additional LAD during TE biopsy cause higher mosaicism rate in blastocysts?

Summary answer: Applying LAD during TE biopsy to create additional zona opening will produce more mosaic blastocysts.

What is known already: In Alpha IVF, laser assisted hatching (LAH) was done on day3 after ICSI for all pre-implantation genetic testing for aneuploidy (PGT-A) cycles. TE biopsy techniques used were laser+pulling (L+P), laser+flicking (L+F) and flicking only (F). At the time of biopsy, an extra step of creating additinonal artificial opening by LAD may be required when (a) blastocyst has very little herniated cells; (b) inner cell mass (ICM) is at the hatching point or biopsy site. Our internal study showed that biopsy using different techniques (L+P, L+F and F) does not affect mosaicism rate.

Study design, size, duration: This prospective study was designed to evaluate the effect of additional LAD during TE biopsy on the mosaicism rate. This study was conducted between 11th March–19th August 2019. Four hundred forty-three (443) patients had undergone oocyte retrieval and blastocyst culture after intracytoplasmic injection (ICSI) was done. A total of 824 hatching blastocyst (BG5) and fully hatched blastocyst (BG6) with at least a Grade A or Grade B TE (Gardner's grading) were included in this study.

Participants/materials, setting, methods: LAH was done on day3 post-ICSI while biopsy was done on day5 and/or day6 for PGT-A. Laser pulse length used during LAH and biopsy was fixed at 400ms. The biopsied blastocysts were classified into 3 groups: (A) BG6 (n=79), (B) BG5 without additional LAD during biopsy (n=713) and (C) BG5 with additional LAD (n=32). The number of biopsied cells ranged from 5-10 cells. Biopsied cells were tested using Next Generation Sequencing (Ion Torrent, USA).

Main results and the role of chance: The mosaicism rates for Group A, B and C were 19.0% (15/79), 23.4% (167/713) and 39.5% (15/38) respectively. Mosaicism rates of Group A and B were comparable (p=0.4807), whilst Group C had significant higher mosaicism rate compared to Group A and B (p=0.0238 and p=0.0319 respectively). The mean age of Group A, B and C were 31.1, 31.4 and 27.1 respectively. The mean age between these 3 groups were not statistically significant (A vs B, p=0.0713; A vs C, p=0.06727; and B vs C, p=0.4408).

Limitations, reasons for caution: Additional LAD during TE biopsy maybe be a confounding variable which affects the mosaicism rate. Moreover, the increase in mosaicism rate could be due to other unknown factors. A larger sample size is needed to confirm the results.

Wider implications of the findings: Based on our study, additional LAD during TE biopsy is not recommended as this may increase mosaicism rate. Biopsy should be done when the blastocyst has more herniated cell or when the ICM leaves the hatching point/biopsy site.

Trial registration number: not applicable

P-149 Calcium ionophores as an aid to surgically retrieved sperms in male factor infertility for increasing cumulative live birth rate

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Study question: Does the addition of calcium ionophores for **artificial oocyte activation(AOA)** help in improving **Cumulative Live Birth Rate** in **surgically retrieved sperms** for male factor infertility?

Summary answer: AOA **significantly improved cumulative live birth rate** in Micro-TESE (**M-TESE**), TESA for non- azoospermia (**TESTICULAR**) and Non-Obstructive Azoospermia(**NOA**)-**TESA** but not in Obstructive Azoospermia (OA)-TESA.

What is known already: The main cause of Total Fertilization Failure after ICSI is thought to be due to oocyte activation deficiency (OAD) because of oocyte-related or sperm-related factors. Studies have shown that artificial oocyte activation (AOA) is helpful in these situations, but is **most effective in couples**

who have clear sperm-related OAD. Oocyte activation, by Phospholipase-C- Zeta **(PLCζ)** present in the sperm, leads to series of events resulting in calcium oscillation, oocyte activation and fertilization. AOA increases the free intracellular calcium thereby mimicking physiologic cell signaling mechanisms that result in oocyte activation and fertilization.

Study design, size, duration: This is a retrospective cohort study done in an academic private ART center, in which patient's records were analyzed, from January 2016 to December 2019 (total 4 years' duration) and all ICSI cycles with surgically retrieved sperms were included (n= 365). Study subjects were divided into 4 groups- M-TESE (n=143), NOA-TESA (n=38), OA-TESA (n=62) and TESTICULAR (n=92). Subdivision was done into cases if AOA was done and control were with conventional ICSI without AOA.

Participants/materials, setting, methods: Method- Immediately after ICSI, in case group (AOA), all metaphase II oocytes were treated with calcium ionophore (GM508- CultActive) for 15 minutes, then thoroughly washed and incubated under standard conditions.

Primary outcome measured was **cumulative live birth rate**(CLBR) and Secondary outcomes were **fertilization rate** (Fert. rate), **Cleavage rate**, **clinical pregnancy rate** (CPR) and **miscarriage rate** (MA). Statistical analysis was performed with Chi-square and Mann-Whitney- U test, with significance at P<0.05. Institutional committee clearance was obtained.

Main results and the role of chance: The **CLBR** was **significantly higher** with **AOA- M-TESE** (55.8% vs 33.3%, *p*- 0.008), **AOA-NOA-TESA** (55.55% vs 15%, *p*- 0.027) and **AOA-TESTICULAR** (62.9% vs 32.3%, *p*- 0.006) group. Fert. rate was significantly higher with AOA-M-TESE (81 ± 0.84 vs 64 ± 0.97, *p*- 0.001), AOA-NOA-TESA (86 ± 0.76 vs 64 ± 0.13, *p*- 0.001) and AOA-TESTICULAR (72 ± 0.12 vs 57 ± 0.11, *p*- 0.001). Cleavage rate, CPR also showed similar significant differences while MA was comparable. However, **significant differences were not observed** in any of the outcome measured in **OA-TESA group between cases and controls** - CBLR (51.6% vs 41.9%, *p*- 0.611), Fert. rate (0.77±0.14 vs 0.75±0.11, *p*- 0.539), CPR and MA, *p*- value > 0.05.

It may be hypothesized that surgically retrieved sperms in cases of NOA or non- azoospermia where TESTICULAR sperms are taken have reduced or absent capacity to cause Calcium oscillations due to deficient or inadequate PLC or there may be some chromatin level abnormalities in these sperms, leading to lesser fertilization and lesser good quality embryos in control group in which AOA was not done.

Limitations, reasons for caution: This study is retrospective in nature. Sibling oocytes were not compared. The study neither looked at obstetrics complication nor the neonatal outcomes. Further studies are required for long term impact on children born from AOA cycles.

Wider implications of the findings: To our knowledge, this is the **first study in the literature** evaluating the efficacy of calcium ionophores for NOA (M-TESE, TESA), OA (TESA) and TESTICULAR sperms. Further research is needed for use of calcium ionophores in cases of unexplained infertility and recurrent implantation failure.

Trial registration number: NOT APPLICABLE

P-150 Does trophectoderm biopsy performed on different blastocyst stages affect the clinical outcome?

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Study question: The objective of this study is to evaluate the effect of trophectoderm (TE) biopsy on different blastocyst stages and its clinical outcome.

Summary answer: Our results showed that TE biopsy significantly reduced the clinical outcome of fully hatched blastocyst. What is known already: TE biopsy is a method widely practiced to harvest cells to determine the chromosomal constitution of a blastocyst, ensuring higher implantation and healthy pregnancies. The effect on clinical outcome after transferring blastocysts biopsied at different blastocysts stages has not been extensively studied.

Study design, size, duration: This retrospective study was conducted from January 2017 until July 2019 at Alpha IVF & Women's Specialists. Following laser assisted hatching on day 3, TE biopsy was performed on unhatched, hatching and fully hatched day-5 blastocysts. A total of 1,020 single euploid blastocysts transfer (SBT) were performed. The average maternal age was 31.7. Implantation rates (IR) were evaluated for all stages of hatching (Unhatched: BG3 & 4; hatching: BG5; fully hatched: BG6).

Participants/materials, setting, methods: Laser assisted hatching (Hamilton Thorne Bioscience, USA) was performed on day-3 and subsequently cultured to blastocyst-stage. Different hatching stages were observed using embryoscope time-lapse system (Vitrolife, Sweden) and were recorded. Day-5 blastocysts with at least BG3BB grade (Gardner's System) were selected for TE biopsy and the biopsied cells were sent for preimplantation genetic testing for aneuploidy (PGT-A) using Next-Generation Sequencing (Life Technologies, USA). All blastocysts were vitrified and warmed using the Cryotec Method (Cryotech, Japan). Main results and the role of chance: All 1,020 blastocysts survived post-warmed (post-warm survival rate= 100%) and were transferred in frozen transfer cycles. TE biopsy performed on unhatched blastocysts showed a comparable IR to hatching blastocysts (60.0% [15/25] and 65.2% [627/961]). While fully hatched blastocysts (44.12% [15/34]) show a significantly lower IR when compared to hatching blastocysts (65.2% [627/961]), no significant difference was seen when comparing unhatched blastocysts to fully hatched blastocysts (60.0% [15/25] and 44.12% [15/34]; p= 0.2949).

Limitations, reasons for caution: The sample size was comparatively smaller in the unhatched and fully hatched group than the hatching group. Further studies with a larger sample size is recommended to ascertain the clinical outcome. Since this is a retrospective study and biopsy was done by different embryologists, the biopsy technique was not controlled. Wider implications of the findings: To achieve higher clinical pregnancy, it is recommended to perform TE biopsy before the blastocysts is fully hatched.

Trial registration number: Not applicable

P-151 Correlation between the first euploid frozen-thawed blastocyst embryo transfer (FBT) and the subsequent euploid FBT outcome originating from the same cohort of oocytes

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Study question: Does the outcome of the first euploid frozen-thawed blastocyst embryo transfer affect the subsequent euploid FBT originating from the same cohort of oocytes?

Summary answer: The clinical pregnancy rate and ongoing pregnancy rate of the subsequent FBT are higher if a clinical pregnancy was attained in the first euploid FBT.

What is known already: Numerous factors including patient, cycle and embryological characteristics affect the outcome of an IVF treatment cycle. There is no data available whether the outcome of euploid FBT has an impact on the outcome of the subsequent euploid FBT of embryos originating from the same cohort of retrieved oocytes.

Study design, size, duration: The study enrolled cycles preimplantation genetic test for aneuploidy (PGT-A) performed between January 2016 and July 2019 at the Bahceci Fulya IVF Center. A total of 1051 patients with single euploid FBT were evaluated and resulted live birth (n=589, live birth rate (LBR): 56%(589/1051)), miscarriage (n=100, miscarriage rate (MR): 14.5% (100/689)) and no clinical pregnancy (n=362, 34,4%, (362/1051)). 159 FBT after the first single euploid FBT originating from the same cohort of oocytes were analyzed.

Participants/materials, setting, methods: Second euploid FBT cycle after first FBT with a clinical pregnancy were compared to frozen-thawed cycles after a without a pregnancy. Logistic regression analysis was utilized to adjust for potential confounders including female age, body mass index, embryo quality, day of embryo frozen, number previous failed attempt, number of previous miscarriage, endometrial thickness, outcome of the first euploid FBT.

Main results and the role of chance: The pregnancy outcome from the first euploid FBT in the study group was resulted live birth (25.1%, (40/159)), miscarriage (15.7%, (25/159)) and no clinical pregnancy (59.1%, (94/159). The pregnancy outcome of the subsequent euploid embryo transfer from the same oocyte cohort was clinical pregnancy rate (CPR): (67.3%, (107/159) ongoing pregnancy rate (OPR) (52.2% (83/159) and MR (22.4%, (24/107)). The CPR in the subsequent euploid FBT was 80% (52/65) among patients who achieved a clinical pregnancy in the first euploid FBT and 58.5% (55/94) of those who did not

(p=0.0045). The OPR in the subsequent euploid FBT was 64.6% (42/65) among patients who achieved a clinical pregnancy in first euploid FBT and 43.6% (41/94) of those who did not (p=0.009). On a multivariate regression analysis, clinical pregnancy in the first euploid FBT was a significant independent predictor for a pregnancy in the subsequent FBT transfer (p=0.003).
Limitations, reasons for caution: The limitation of the study is in the retrospective nature of the study. As the PGT-A strategy significantly decreases number of transferable embryos, the sample size of the study is limited.
Wider implications of the findings: Identifying predictive factors for the success of euploid FBT is important. These can help physicians while counseling patients regarding the outcome of the previous euploid FBT.
Trial registration number: NA

P-152 Morphokinetic and maternal profiles of embryos derived from centrally granulated oocytes vary with their ability to implant

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Study question: Does oocyte central granularity (CG) impact embryo morphokinetics and does this change with embryo implantation ability and maternal profile?
Summary answer: Oocyte CG slows fertilization and cleavage morphokinetics in overall derived embryos, but not in those capable to implant, an ability associated with lower maternal age.
What is known already: Oocyte morphology is easily accessible after pre-ICSI oocyte denudation, but the implications of morphological alterations for oocyte developmental competence are not entirely known. The presence of a centrally located granular area in the ooplasm was previously associated with alterations in the actin cytoskeleton and meiotic spindle, both potentially affecting meiosis completion, fertilization dynamics and embryo mitotic divisions. In fact, we have recently reported lower fertilization rates and delayed pronuclei fading and first cleavage associated with the presence of CG in oocytes subjected to ICSI.
Study design, size, duration: Retrospective analysis including 1378 control ICSI cycles providing only morphologically normal oocytes (1225 patients) and 220 CG cycles (201 patients) providing normal and CG oocytes, from July 2014 to March 2020. Morphokinetic parameters were compared between embryos from control and CG oocytes, as well as among embryos from control oocytes reaching implantation (C-I) and embryos from CG oocytes achieving (CG-I) or not (CG-NI) implantation. Maternal profiles were compared between CG-I and CG-NI.
Participants/materials, setting, methods: Oocytes were recovered from patients after controlled ovarian stimulation and ovum pick-up. Following ICSI, embryo culture was performed in a time-lapse incubator with annotation of time of pronuclei fading (tPNf) and cleavage times t2, t3, t4, t5 and t8. Morphokinetic data were retrospectively coupled with implantation outcomes of single transfers and of double transfers achieving double or no implantation. Differences were assessed with Chi-square and Kruskal Wallis tests.
Main results and the role of chance: Patients providing CG oocytes (n=201) presented higher maternal age (37.4 ± 4.4 vs. 36.7 ± 4.3; p=0.005), higher basal FSH (8.52 ± 3.7 vs. 7.62 ± 2.8 IU/L; p=0.002) and lower AMH levels (2.2 ± 2.2 vs. 2.9 ± 3.1 ng/mL; p<0.001) compared to control patients (n=1225). Morphokinetic parameters from tPNf to t4 were faster in embryos derived from oocytes with normal morphology (control; n=6947) compared to embryos derived from CG oocytes (n=382; tPNf: 24.0 ± 3.8 vs. 24.6 ± 3.6; t2: 27.0 ± 4.3 vs. 27.6 ± 4.1; t3: 37.0 ± 5.7 vs. 37.4 ± 5.7; t4: 39.4 ± 6.4 vs. 40.1 ± 6.2 hours; p<0.05). In addition, CG-NI (n=103) embryos were slower than CG-I (n=13) and C-I (n=226) embryos for tPNf, t2, t3, t4 and t8 (p<0.05), while CG-I did not differ from C-I embryos (p>0.05; tPNf: 22.4 ± 2.6 vs. 22.0 ± 2.5; t2: 24.9 ± 2.7 vs. 24.6 ± 2.8; t3: 36.0 ± 3.5 vs. 35.4 ± 3.0; t4: 36.7 ± 3.5 vs. 36.7 ± 3.6 hours, for C-I and CG-I, respectively). Finally, patients providing CG-I embryos (n=10) were younger than those providing CG-NI embryos (n=65; 31.3 ± 4.6 vs. 38.0 ± 3.9; p<0.05).
Limitations, reasons for caution: Our study is subjected to the intrinsic limitations of a retrospective analysis, the results presented could have been affected by variables that are uncontrolled for. Other studies are necessary to assess the impact of CG on clinical outcomes.
Wider implications of the findings: The findings indicate that early developmental morphokinetics and maternal age constitute valid parameters for the decision of whether to transfer CG-derived embryos, as well as for the transfer prognosis.
Trial registration number: not applicable

P-153 Comparison outcome of vitrified human embryos stored in vapor phase liquid nitrogen (LN2) and direct LN2

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Study question: Is vapor cryopreserved LN2 storage beneficial for clinical outcomes of vitrified human embryos that are frozen compared to vitrified human embryos having direct contact with LN2.
Summary answer: There are no significant differences compared to clinical outcomes of human embryos stored in LN2 vapor and direct store in LN2.
What is known already: There has been concerned about potential cross-contamination and biohazard issues of embryos for long term storage using direct LN2. This study aimed to compare clinical outcomes of human embryos transfer between vapor phase and liquid LN2.
Study design, size, duration: The embryo has undergone vitrification for long term storage with vapor or direct contact in LN2. After the thawing of the embryo, we checked on the survival rates. We transferred only one or two embryos per patient and kept analyzing the implantation and pregnancy rates
Participants/materials, setting, methods: This retrospective study was carried out from January 2018 to December 2019 with 3272cycles 4713embryos; vitrified for long term storage in vapor phase or direct contact with LN2. We compared the clinical outcomes of frozen embryo transfer cycles using vitrified for long term storage in vapor phase and direct contact with LN2. Clinical outcomes monitored were embryo survival, subsequent implantation and pregnancy after single or double embryo transfer
Main results and the role of chance: A total of 4713 fertilized human embryos are vitrified and then stored in LN2 vapor (n=2520 cycles) or direct contact LN2 (n=752 cycles). The study showed that the blastocyst stored in vapor able to retain full development. Survival was 97.8% (vapor) and 97.6% (direct contact LN2), and the vapor storage of human embryos had no significant difference in survival rates after a long term storage. For single blastocyst transfer, pregnancy and implantation rates were 51.5%, 52.4% in vapor, 54.6%, 54.9% in direct LN2; respectively (p=NS). In double blastocyst transfer, the pregnancy and implantation rates were 61.8%, 42.0% in vapor and 64.7%, 44.5% in direct LN2; respectively (p=NS). There were also no significant differences between two groups.
Limitations, reasons for caution: The study showed that the blastocyst stored in vapor can retain full development. A vapor storage system thus is safe and effective for long term vapor storage of vitrified human embryos.Within the limits of this study, there was no detection of an adverse effect of vapor storage.
Wider implications of the findings: Vapor storage systems thus represent a useful alternative for safe and effective long-term storage of vitrified human embryos that can avoid cross contamination chances from having direct contact with LN2.
Trial registration number: not applicable

P-154 The role of the X Chromosome in early human embryo metabolism

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Study question: What role does the X chromosome play in early embryo metabolism? Does X chromosome copy number contribute to sex differences in early embryonic metabolism?

Summary answer: Chromosome X contains several metabolism-related genes that are expressed prior to X-inactivation, suggesting that their dosage plays a role in sex-biased regulation of embryo metabolism.
What is known already: Published reports indicate that sex differences in preimplantation embryo metabolism exist across mammalian species, including humans. Two observations supporting this are that male embryos reach blastocyst stage earlier than their female counterparts, and that glucose uptake and processing is thought to be higher in female compared to male embryos. It has been hypothesized that these differences reflect the location of the metabolism gene *G6PD*, the rate limiting enzyme in the Pentose Phosphate Pathway, on Chromosome X.
Study design, size, duration: This study is a reanalysis of publicly available RNA-seq data, including 1176 single cells from 59 blastocysts (24 E5, 18 E6, 17 E7) published in one study (Petropoulos et al 2016).
Participants/materials, setting, methods: Cells were subjected to a digital karyotype inference algorithm and aneuploid samples were removed from the dataset. Sex differential gene expression analyses (DE) were then performed in euploid trophectoderm cells (TE; 233 XY from 16 embryos and 180 XX cells from 12 embryos). Cell numbers from ICM were too sparse to compare.
Main results and the role of chance: Analysis of XX and XY TE revealed 618 significantly differentially expressed genes (DEGs; 507 upregulated in XX cells, and 111 upregulated in XY cells). These genes are spread across autosomes and sex chromosomes. Interestingly, *G6PD* is not significantly more highly expressed in XX cells.

Gene Ontology (GO) analysis of the XX-biased DEGs revealed a transcriptional sex bias in metabolism-related GO categories, including "mitochondrial ATP synthesis coupled electron transport", and "respiratory chain complex I".

Gene-level assessment revealed that the drivers of these enrichments are spread across the genome, but 28/64 reside on Chromosome X (hypergeometric p-value = 5.984473e-27), including *NDUFA1*, *NDUFB11*, and *COX7B* (components of the electron transport chain), and *SLC25A5* (an ATP/ADP transporter involved in maintaining mitochondrial membrane potential). This indicates a direct role for multiple X-linked genes in sex-biased regulation of embryo metabolism.

Metabolic genes that are not sex-biased are distributed across the genome, with no significant enrichment on Chromosome X (76/266, hypergeometric p-value=0.607). Together, these data indicate that GO metabolic term X enrichment is a feature of sex-biased expression and not due to an accumulation of metabolism-related genes on the X.
Limitations, reasons for caution: This analysis draws on publicly available data, and thus we are unable to perform orthogonal validation of karyotype calls. Additionally, while the initial dataset is large, the quality-filtered dataset (euploid XX and XY TE) is small, and single cell data is infamously variable. Further data collection is required.
Wider implications of the findings: Our analysis of sex-biased gene expression in early human embryos suggests a more important role for the X chromosome in modulating sex biases in early embryo metabolism than previously recognized. This study provides insight into the mechanisms underlying the development of metabolic sex differences throughout the lifespan.
Trial registration number: NA

P-155 Oocyte recovery 39 hours (from 39h to 41h) after administration of follicular maturation trigger does not affect clinical results

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Study question: What is the clinical outcome of oocytes recovered after 39 hours from ovulation inducing drug administration?
Summary answer: Oocytes obtained after 39 hours from follicular maturation triggering are equally viable to those obtained at the standard time of 36 hrs.
What is known already: In the clinical setting of ART, ovum pick-up (OPU) is generally performed around 36 hours after the administration of ovulation inducing drugs (OID). However, there are cases where OPU cannot be performed at this time often due to long operating lists. As the time elapsed between the administration of ovulation inducing drugs and OPU becomes longer, there is a concern about time-related oocyte aging. Nevertheless, there are few reports of clinical results of OPU after 36 hours from OID.
Study design, size, duration: We conducted a review of 1187 cycles and 1951 patients in which OPU and embryo transfer was performed in 2017-2018. All cycles underwent a 'freeze-all' of embryos and the transfer cycle was in the thawed embryo transfer cycle for all cases.
Participants/materials, setting, methods: The time from the administration of OID to the end of OPU was divided into 36h group and over 39h group and the MII and normal fertilization rate of oocytes obtained from OPU after ovarian stimulation were compared. After confirmation of fertilization, the D3 good-quality embryo and the D5 and 6 good-quality blastocyst rates of embryos that continued to be cultured and the pregnancy and miscarriage rates of cleavage-stage embryos and blastocyst transfers were compared.
Main results and the role of chance: The MII rate in the 36h and >39h groups was 78.1% vs. 80.0%, and the normal fertilization rate was 77.9% vs. 78.1% (ICSI) and 65.4% vs. 67.6% (Conventional-IVF). The D3 good-quality embryo rate (good-quality embryos are embryos with less than 5% fragmentation in 7-9 cells and compaction with more than 50% adhesion between split spheres) was 21.8% vs. 25.3%, the D5 good-quality blastocyst rate (at least 3BB according to Gardner classification) was 33.6% vs. 40.1%, and the D6 good-quality blastocyst rate was 31.1% vs. 37.5%, all of which were not significantly different. The pregnancy rate for cleavage-stage embryo transfer was 26.6% vs. 6.7%, and the miscarriage rate was 25.3% vs. 42.9%, both of which were not significantly different. The pregnancy rate for blastocyst transfer was 45.4% vs. 50.0%, and the miscarriage rate was 22.2% vs. 20.0%, both of which were not significantly different. (The significance difference test was a χ-square test)
Limitations, reasons for caution: The study was a retrospective study.
Wider implications of the findings: Even if OPU is conducted after 36h of the administration of OID, to the extreme range of 39h-41h, oocyte aging does not seem apparent and pregnancy outcomes are similar to the standard time interval of 36 hours.
Trial registration number: 'not applicable'

P-156 Automatic pronuclear detection based on deep learning technology has clinical utility

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Study question: Does the performance of an automatic pronuclear detection system based on deep learning technology have clinical utility?
Summary answer: Output results for 2PN detection using the automatic system powered by deep learning technology has clinical utility.
What is known already: In order to establish a more objective embryo evaluation system, we have been developing an automatic pronuclear detection system that utilizes deep learning technology based on Time- Lapse (TL) images. We have previously reported that the accuracy of pronuclei detection was improved by introducing an analysis method using 11 slices in the Z axis. In this study, we evaluated the potential clinical practicality of the automatic pronuclear detection system.
Study design, size, duration: Embryos clinically evaluated between May 2018 and December 2019 by embryologists were chosen for this study. We prepared for analysis TL videos of 995 embryos that had been evaluated as having 0, 1, 2, and 3PN.
Participants/materials, setting, methods: Part1:We compared the outputs of the automatic pronuclear detection system with these embryologists(three junior embryologists (1a), three intermediate embryologists (1b),and three senior embryologists (1c)) who had judged the pronuclei number from TL videos from 40 embryos each having 0,1,2,and 3PN.

Part2:The automatic pronuclear detection system determined the pronuclei number from the TL videos of 955 embryos scored as either 1,2,and 3PN,(different from those used in Part1),and the detection rate for 2PN was calculated.
Main results and the role of chance: Part1: The sensitivities for embryologist groups 1a),1b),1c) and the automatic pronuclear detection system were 80.0%,100%,100%,100% for 2PN, 60.0%,83.3%,86.7%,100% for 0PN, 46.7%,80.0%,86.7%,10.0% for 1PN, and 73.3%,96.7%,96.7%,10.0% for 3PN.

Part2: The precision for 2PN by the automatic pronuclear detection system was 99%.

Limitations, reasons for caution: In order to further improve the performance of the automatic pronuclear detection system, further adjustment of the algorithm and more training images will be utilised.

Wider implications of the findings: The detection of 2PN by the automatic pronuclear detection system was highly reliable, and the performance of the system was comparable to that of embryologists. These first results are reassuring and support the clinical use of the system as a further aid for embryologists, in routine laboratory practice.

Trial registration number: 'not applicable'

P-157 The pregnancy potential of embryos cryopreserved and thawed twice: A case-control study

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Study question: What are the pregnancy and perinatal outcomes of twice-cryopreserved embryos compared to once-cryopreserved embryos?

Summary answer: Transfers of twice-cryopreserved embryos result in similar live birth rates (LBR) and perinatal outcomes compared to transfers of once-cryopreserved embryos.

What is known already: Repeated cryopreservation of viable surplus embryos in frozen embryo transfer (FET) cycles is a potential method to increase the cumulative pregnancy rate and reduce the risks related to multiple pregnancies. Currently, evidence on the safety and success of repeated cryopreservation is limited. Existing data from a few studies indicate that the vitrification of previously slow-frozen or vitrified embryos does not negatively impact pregnancy outcome, and no long-term health consequences in neonates have been reported. However, due to the limited number of reported pregnancies and children, more studies are needed.

Study design, size, duration: This retrospective register-based case-control study included FETs (n=2834) performed at the University Hospital of Turku and the Central Hospital of Central Finland, Finland, between January 2012 and December 2019. The case group consisted of twice-cryopreserved FETs (n=89), and the control group consisted of once-cryopreserved FETs (n=304). The matching criteria were embryonic age at transfer and female age category of less or over 35 years.

Participants/materials, setting, methods: All the FETs in the case group and 86% in the control group were single-embryo transfers (p=<0.001). The first cryopreservation was performed by slow freezing or vitrification (cases 58% vs 42% and controls 40% vs 60%, p=0.002, respectively). The re-cryopreservation method was vitrification. Mixed effects logistic regression was used to analyse the pregnancy outcomes, and a linear mixed model was used to analyse neonatal weight, adjusting for gestational age, neonatal sex, parity and BMI.

Main results and the role of chance: The survival rate of the twice-cryopreserved embryos was 92.2 % (94/102), and 93.7% (89/95) of the planned FETs could be carried out. FET was performed with D3-4 embryos in 17 cases and 68 controls and with D5-6 embryos in 72 cases and 238 controls. The rates of live birth, clinical pregnancy and miscarriage in the case and the control groups were comparable (27.0% vs 31.9%, p=0.35; 31.4% vs 36.8%, p=0.35 and 4.5% vs. 3.9%, p=0.77, respectively). No difference was seen in the preterm delivery rate (cases 4.2% vs controls 10.3%, p=0.69). Twenty-five children were born in the case group and 100 in the control group. No differences in birth weights were detected between the groups (3730 g, upper and lower quartiles 3500 g and 4050 g, vs 3490g, upper and lower quartiles 3150 g and 3900 g, p=0.28), and, in the case group, all the newborns' birth weights were appropriate for gestational age. There were no congenital malformations among the newborns in the case group. In the control group, there was one pregnancy termination due to aneuploidy, one case of undescended testicles, one child with a hypoplastic aortic valve without stenosis and one child with craniosynostosis.

Limitations, reasons for caution: This study was retrospective, and the small sample size limits interpretation of the results. FET has been demonstrated to increase the risk for fetal macrosomia and gestational hypertension/pre-eclampsia. Whether repeated cryopreservation enhances these effects or influences neonatal health in the long term needs further investigation.

Wider implications of the findings: Acceptable LBR and neonatal outcomes may be expected after transfer of twice-cryopreserved embryos. Also, the survival rate is high. To avoid embryo wastage or transfer of multiple embryos, good quality surplus embryos from FET cycles may be considered for repeated cryopreservation by vitrification.

Trial registration number: not applicable

P-158 Assisted Hatching on D+3 in order to facilitate trophectoderm biopsy in blastocyst for PGT-A is not advisable in all patients

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Study question: Is it useful or beneficial to perform Assisted Hatching (AH) on D+3 previously to biopsy for PGT-A on blastocyst stage on D+5?

Summary answer: The routine use of AH on D+3 to facilitate the embryo biopsy on D+5 could negatively influence the development of the embryos to blastocyst stage.

What is known already: The blastocyst stage is the optimal stage for performing biopsies for PGT-A, which has been reported as a key factor determining the growing clinical application of this strategy worldwide. For trophectoderm (TE) biopsy, laser-assisted drilling is used to create a zona opening on D+3 or D+5 of development. The method of zona opening on D+3 allows some of the TE cells to herniate during blastocyst formation and expansion, which facilitates the biopsy process. However, this method may result in herniation of inner cell mass cells instead of TE or maybe could affect the development of the embryo to blastocyst stage.

Study design, size, duration: A total of 100 PGT-A cycles were performed in 2019 and 2020. In 78 of them laser-assisted drilling was used to create a zona opening on D+5 only in those embryos which arrived to blastocyst stage for TE biopsy (Group No-AH). In 22 cycles the same drilling was achieved on D+3 in all embryos, independently of their quality (Group AH). The average of embryos per cycle in each group was 5 and 4.3 respectively.

Participants/materials, setting, methods: A total of 100 PGT-A cycles coming from 65 patients were studied. The average of the age of the patients was 40.83 (SD 3.45) in the group No-AH vs 42.18 (SD 3.42) in the Group AH (p= 0.108), so the age was not a determining factor for the development of the embryos. We analyzed by 2 test differences between groups on fertilization rates, number of embryos, development to blastocyst stage, euploidy and pregnancy rates.

Main results and the role of chance: The fertilization rate was 74.79% (No-AH group) and 68.53% (AH group) with no significative statistical differences (p=0.12).

In the No-AH group, the TE biopsy was performed on D+5 in 63 cycles (81%). In the AH group, 41% of cycles didn't reach the blastocyst stage, obtaining statistical differences between groups (p=0.035). We found also significant differences in the number of cycles with biopsied blastocyst when we had 1 to 6 embryos/cycle on D+3 between groups (p=0.002), without obtaining any blastocyst to be diagnosed in 53% of the cycles in AH group vs 27% in No-AH group. When the number of embryos on D+3 per cycle was > 6, at least 1 embryo reached the blastocyst stage in both groups, although this number was higher in No-AH group. The rate of biopsied blastocysts was significantly higher in the No-AH group compared to the AH group (46.61 vs 34.69) with a p=0.031.

The rate of euploid embryos analyzed was 23.30% in the No-AH group compared to 29.41% in the AH group, although no significant differences were found (p=0.44) between groups.

In the No-AH group, a clinical pregnancy rate of 52.94% was obtained (n=34) vs 50% in the AH group (n=4) (p=0.91).

Limitations, reasons for caution: We have recently started to perform AH on D+3, so the number of cases is smaller than No-AH group. We use a time lapse incubator in all cases, so in the No-AH the culture dish is changed, disturbing the stable incubation environment, while in the other group it is not.

Wider implications of the findings: The use of AH on D+3 in order to facilitate the TE biopsy on D+5 could affect negatively the development of the embryos to blastocyst stage. Its routine use should be avoided based on laboratory workload, mainly if the patient has less than 7 embryos at D+3.
Trial registration number: Not applicable

P-159 Slow-growing embryos should be frozen on day 5

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Study question: What is the live birth rate after frozen embryo transfer (FET) of slow-growing embryos frozen on day 5 (D5) or on day 6 (D6)?
Summary answer: The live birth rate after single FET is significantly higher for slow-growing embryos frozen on D5 compared to those frozen on D6.
What is known already: Most data on the outcomes of blastocyst transfer stem from studies that evaluate fresh transfer from normal growing D5 blastocyst ET. However not all embryos will begin blastulation nor reach the fully expanded stage by D5; those are the slow-growing embryos. Studies that compare D5 to D6 embryos in FET cycles show contradictory results. Some have reported higher clinical pregnancy rates after D5 FET, while others have reported similar outcomes for D5 and D6 cryopreserved blastocyst transfers. There is a lack of evidence regarding the best approach for vitrifying embryos that exhibit a slow developmental kinetic.
Study design, size, duration: This retrospective cohort study included 821 single FET of slow-growing embryos frozen on D5 or D6, belonging to patients undergoing in vitro fertilization with donor oocytes between January 2011 and October 2019, in a single fertility center. The origin of blastocysts was either supernumerary embryos after fresh embryo transfer or blastocysts from freeze-all cycles. All embryos were transferred 2- 4h after thawing.
Participants/materials, setting, methods: We compared reproductive outcomes of slow-growing embryos frozen on D5 versus (n=442) slow-growing embryos frozen on D6 (n=379). D5 group consisted in embryos graded 0, 1, 2 of Gardner scale and frozen on D5. Similarly, D6 group consisted in embryos graded 3, 4, 5 of Gardner scale (blastocyst stage) and frozen on D6. Differences in pregnancy rates between study groups were compared using a Chi2 test. A p-value <0.05 was considered statistically significant.
Main results and the role of chance: Baseline characteristics were comparable between study groups. Overall, mean age of the woman was 42.3±5.4 years old; donor sperm was used in 25% of cycles, and it was frozen in 73.2% of cycles. Pregnancy rates were significantly higher when transferring slow D5 embryos compared to D6 for all the pregnancy outcomes analyzed: biochemical pregnancy rate was 27.7% vs 20.2%, p<0.016; clinical pregnancy rate was 17.5% vs 10.2%, p<0.004); ongoing pregnancy rate was: 15.7% vs 7.8% (p< 0.001); live birth rate was: 15.4% vs 7.5%, (p<0.001). These results suggest that when embryos exhibit a slow development behavior (not reaching full blastocysts at D5), waiting until D6 for blastulation and expansion does not improve clinical outcomes. Vitrification at D5 will should the preferred option in cases where the oocyte is assumed of high quality
Limitations, reasons for caution: The retrospective design of the study is its main limitation. Also, morphology as sole selection criterion for transfer. However, blastocyst morphology is a very good predictor of implantation and pregnancy, and a good indicator of the embryo's chromosomal status (higher euploidy rate in higher morphological quality blastocysts).
Wider implications of the findings: These results can help to the standardization of laboratory protocols. As the decision of vitrifying slow developing embryos on D5 or D6 is made by the laboratory team or by the gynaecologist in agreement with the patient, having an evidence based strategy simplifies patient counselling and decision making.
Trial registration number: not applicable

P-160 Sibling oocytes cultured in a time-lapse versus benchtop incubator: limited exposure of embryos outside the incubator improves outcomes

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Study question: Does the limited exposure of embryos outside the incubator, during evaluation and changeover, have an impact on the blastocyst development, blastocyst quality and euploid outcomes?
Summary answer: Exposure of embryos outside the incubator, negatively impacts the number, quality and euploidy rate of day 5 blastocysts.
What is known already: The laboratory environment with its culture conditions is one of the crucial elements of the delicate equation to a successful ART outcome. It has been shown that increased fluctuations in the culture conditions have a considerable impact on the number of blastocysts obtained and cycle outcomes. Compared to conventional benchtop incubators, Time Lapse Technology (TLT) incubators capture images of the embryo and allow morphologic and morphokinetic assessment without disturbance during incubation. Several studies have been published comparing the efficiency, safety and outcome performance between conventional and TLT incubators, however, none of them explored the euploid outcomes.
Study design, size, duration: An observational sibling oocyte study was performed at ART Fertility Clinics, Abu Dhabi between March 2018 and April 2020 and included data of 796 mature oocytes injected from 42 stimulation cycles. Sibling oocytes were randomly split between 2 different incubators: 12 oocytes were assigned to the twelve wells of the EmbryoscopeTM (ES) and the remaining oocytes were cultured in a conventional benchtop incubator, G185 K-System (KS).
Participants/materials, setting, methods: Embryos from patients with primary or secondary infertility, who underwent ovarian stimulation for ICSI and PGT-A through NGS on trophectoderm biopsies, were eligible. All patients had at least 16 fresh mature oocytes, randomly allocated to two different incubators after ICSI: 503 (63.2%) oocytes were cultured in ES and 293 (36.8%) in KS. The fertilization, cleavage, useable blastocyst and euploid rates, as well as embryo/blastocyst qualities were assessed to evaluate each incubator's performance.
Main results and the role of chance: The fertilization and cleavage rates were similar between incubators. Total useable blastocyst rate (64.8% *vs* 49.6%, *p*<0.001) was significantly higher for embryos cultured in ES, mainly due a higher percentage of blastocysts biopsied on day 5 in ES (67.8% *vs* 57.0%, *p*=0.037), with improved quality (p=0.008). There was no difference in the total euploid rate between ES and KS (59.9% *vs* 50.4%, *p*=0.314), but a significantly higher euploid rate was seen for blastocysts cultured in ES and biopsied on day 5 (63.5% *vs* 37.4%, *p*=0.001). Day 3 embryo quality and total biopsied blastocyst quality was not different between incubators. No difference was observed in the total useable blastocyst development from good (p=0.0832) and poor (p=0.112) quality day 3 cleavage stage embryos. However, when stratifying according to the day of blastocyst development, poor quality embryos on day 3 showed superior blastocyst formation on day 5 when cultured in ES (64.1% vs 39.1% for day 5 and 35.9% vs 60.9% for day 6, p=0.005). Accordingly, blastocyst formation from poor quality embryos on day 3, was shifted to day 6 for embryos cultured in KS. This difference in the day of blastocyst development was not observed for good quality cleavage stage embryos (p=0.917).
Limitations, reasons for caution: The current observational study needs confirmation in a prospective trial and should also include the implantation potential of the euploid blastocysts, which was not followed in the current study. A good prognosis population (≥16 mature oocytes) was studied and may not reflect the outcomes in patients with lower oocyte numbers.
Wider implications of the findings: This work builds evidence to the solid introduction of the TLT incubators to the clinical routine, as the reduced exposure of embryos outside the incubator – and hence decreased stress - improves the blastocyst development.
Trial registration number: NA

P-161 Is enough the staff in your lab?

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Study question: Must be all the activity made in *in vitro* fertilization (IVF) laboratories keep in mind to size its staff?
Summary answer: To create a staff calculator based on number of cycles carry on, assisted reproduction techniques, quality controls, administration management, weekend duties, labour regulations and holidays.
What is known already: In a bibliographic search about staff in human reproduction labs there is no mention about de number of embryologists recommended for every cycle done. Only that it will be according to the workload. Other guidelines establish that every embryologist could assume 150 IVF cycles/year. However, here is a downward tendency in the work that an embryologist can assume. Alikani established a maximum of 100 cycles/year for every embryologist (Alikani *et al,* 2014).
Study design, size, duration: Seven senior embryologists working in different IVF centres, three public and 4 privates, take part lead in this Multicentre study during 2019 and 2020. We made a survey to create a calculator for staff using the mean time spent in every lab by each embryologist of the centre to do any IVF procedure and measured three times each one.
Participants/materials, setting, methods: Different lab procedures and activities related with quality control, time spent to do them, and witnessing were included in the survey. For the calculations it was considered an embryologist with a full-time contract working 1744 hours / year according to current labour agreement in Spain.

The times included in the calculations for each task were those corresponding to the 95th percentile. For the calculation, the program used was Microsoft Office Excel.
Main results and the role of chance: In the IVF laboratory many gametes and embryos from different couples are manipulated daily. The maintenance of traceability could be affected by not having the right staff and lead to dramatic consequences for the patients and the centre.

Workload or overload caused by non-suitable staff number also affects the embryologist having a direct impact on his health.

The results of the survey carried out showed the time needed by embryologist to perform the different procedures necessary for an IVF treatment, being a classic IVF cycle (8.11 hours), also taking into account the time spent in managing documentation, preparing the cycle and databases. An ICSI with Time lapse needs 10.27 hours and an ICSI-PGD cycle 13.91 hours. To all off this, 1.81 hours should be added for every vitrification support needed and the time to control more than 200 critical steps, including equipment control and culture parameters.

The time spent in semen analysis (including managing documentation, cycle preparation and databases) or intrauterine insemination with a partner sperm was 2.7 hours. For donor sperm an additional hour for the management involved is required. The time required to perform and cryopreserve a testicular biopsy and seminal cryopreservation was 4 and 3.7 hours, respectively.
Limitations, reasons for caution: The study was made taking account of Spanish regulations, quality standards and recommendations and should be adapted to the foreigner's regulations**.** Wider implications of the findings: New advance staff calculator allows laboratories estimate minimum number of embryologist necessary for a particular public or private laboratory without compromise neither security nor success in their results. Nevertheless, we recommended a minimum of two qualified embryologists in every lab, whatever it was the workload.
Trial registration number: none

P-162 Laser-assisted collapse of blastocysts prior to vitrification improves clinical outcomes

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Study question: What is the effect of artificial laser-assisted collapse before vitrification on pregnancy and implantation rates after transfer of vitrified-warmed blastocysts?
Summary answer: The artificial shrinkage by laser-induced collapse before vitrification significantly increased the implantation and clinical pregnancy rates after single thawed embryo transfer.
What is known already: Freeze all, cycle segmentation and, in general, single embryo transfer (SET) strategies (for example trophectoderm biopsy-based aneuploidy testing) have targeted blastocysts vitrification as the best option for reproductive practice worldwide. Artificial shrinkage seems to be a pre-vitrification parameter associated with an increased embryo survival after warming and implantation rate. However, the available medical evidence shows controversial results with only a limited number of prospective studies assessing the subject.
Study design, size, duration: This prospective cohort study evaluated 394 women who underwent a frozen blastocyst transfer at Instituto Bernabeu between July and December 2020. All patients were prepared with substitutive cycle and received single blastocyst embryo transfers.
Participants/materials, setting, methods: Before embryo vitrification on day 5 of development, some expanded and/or early hatching blastocysts (A/B ASEBIR categories) were artificial laser-assisted collapsed. (n=83, study group). 311 embryos of the same quality and day of development were not collapsed (control group). We compared the embryo survival rate, clinical, implantation and miscarriage rates between groups. The statistical analysis was performed using SPSS (version 20.0).
Main results and the role of chance: The two groups were comparable in terms of maternal age (39.79 ± 3.83, control group; 40.21 ± 4.45, study group; p=0.341). Embryo survival rate resulted in 100% in both groups.

Regarding clinical outcomes, collapsed blastocysts significantly increased the positive pregnancy test and the clinical pregnancy and implantation rate compared to the control group, respectively (positive test: 69,9% vs 43,4%, p=0.000018, odds ratio (OR)= 3.02 [95% CI 1.80–5.08]; clinical pregnancy and implantation: 56,6% vs 35,4%, p=0.000041, OR= 2.39 [95% CI 1.46–3.90]). The miscarriage rate was not affected by the blastocyst collapse effect (23,6% in the control group vs 27,6% in the study group, p=0.593, OR= 1.23 [95% CI 0.57-2.68]).
Limitations, reasons for caution: This is a non-randomized controlled study. Additional RCTs are warranted to corroborate our findings.
Wider implications of the findings: Considering the large number of blastocyst vitrification cycles that are carried out worldwide, artificial laser-assisted collapse before vitrification has the potential to increase the clinical results in benefit of many patients.
Trial registration number: Not Applicable

P-163 The presence of trophectodermal vesicles on expanded ICSI-derived blastocysts is a good predictor of implantation

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Study question: Is the implantation rate (IR) higher in blastocysts with trophectodermal vesicles (TVs) compared to blastocysts without TVs or euploid blastocysts with unknown spontaneous hatching status?
Summary answer: The blastocysts with TVs demonstrate significantly higher IR in comparison to blastocysts without TVs or euploid blastocysts with unknown spontaneous hatching status.
What is known already: After ICSI spontaneous hatching mainly occurs by trophectoderm cell herniation via a small slit in the zona pellucida. At the beginning of this process, TVs are formed on the outside of the zona pellucida. It was previously shown that the clinical pregnancy rate was similar after the transfer of expanded blastocysts and expanded blastocysts with TVs. But another study showed that transfer of blastocysts of more advanced hatching stages yields better pregnancy rates than expanded blastocyst transfer. It remains unclear whether there is an association between the presence of TVs and IR in the transfers of single vitrified blastocysts.

Study design, size, duration: This retrospective cohort study was conducted from October 2018 to November 2019 and included 477 transfers of a single vitrified blastocyst. Cases were divided into 3 groups. Group 1 included transfers of blastocysts without TVs and with assisted hatching (AH). Group 2 contained the transfers of blastocysts at TVs stage of spontaneous hatching and without AH. Group 3 consisted of transfers of the euploid blastocysts with AH performed and unknown spontaneous hatching status.
Participants/materials, setting, methods: The age of women was between 21 and 39. Embryo transfers following oocyte donation programs were excluded from the study. This study included only transfers of the ICSI-derived fully expanded blastocysts with top-graded inner cell mass and trophectoderm. AH was performed using laser Saturn 3. The primary outcome was the implantation rate. Statistical analysis was performed using Pearson's chi-square test and likelihood ratio test. Preimplantation genetic testing for aneuploidy (PGT-A) was performed by next-generation sequencing.
Main results and the role of chance: The number of cases in groups 1,2 and 3 was 133, 49, and 295, respectively. The average age in the groups was about 32.5 and did not differ between groups. The implantation rate in group 3 with PGT-A was 60% (177 out of 295), which was insignificantly higher compared to group 1 - 55% (73 out of 133) (p = 0.34). In group 2, the implantation rate was 76% (38 out of 49), which exceeded significantly the outcomes in groups 1 and 2 (p = 0.016). Thus the transfer of expanded blastocyst with TVs gives higher IR in comparison to expanded blastocyst. Therefore TVs could be utilized as a morphological marker for embryo selection. Furthermore, according to obtained results the presence of TVs on nontested blastocysts predicts implantation better than euploidy does in blastocysts with unknown spontaneous hatching status.
Limitations, reasons for caution: This is a retrospective nonrandomized study with its inherited limitations.
Wider implications of the findings: Based on the results of the study embryo selection practice could be optimized. To maximize the outcomes of PGT-A programs embryo culture and biopsy workflow could be modified to allow collecting data on spontaneous hatching and TVs presence before performing the biopsy.
Trial registration number: not applicable

P-164 Mulitcentre derived time lapse algorithms developed using 6228 transferred embryos with known birth outcome incorporating novel morphological and morphokinetic markers

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Study question: Can incorporation of novel markers of morphology with known temporal events successfully rank embryos to enable prediction of propensity for live birth?
Summary answer: Incorporation of variables for trophectoderm and morula grading demonstrably enhanced the model to rank embryos in order of potential for live birth.
What is known already: Models built using morphokinetic markers of development are widely used to rank embryos within a cohort. Such models include defined temporal parameters which are closely related to morphological grade. However, morphological grading by an embryologist is subjective and is not strongly correlated to outcome. Combining with defined kinetic events has been suggested to improve prediction of outcome.
Study design, size, duration: Data from 6228 known live birth outcome embryos from 8 UK clinics between 2011 – 2018 were investigated using an exploratory approach to identify novel markers of development.
Participants/materials, setting, methods: Five significant variables were defined, a derivative of time to start of blastulation; a derivative of trophectoderm grade; a kinetic variable utilising t3, t4, t5 and t8; an interval variable of tB-tSB and a variable based on novel morula classification. To maximise the output, a proxy value was derived for missing datapoints. The model was built using logistical regression and validated using fivefold cross validation with the data split as 80% training and 20% test.
Main results and the role of chance: An algorithm was developed including the five significant variables identified with an AUC of 0.685 demonstrating reliable prediction of live birth. Without morphological variables, the AUC was 0.674 demonstrating the improvement in the prediction value by including the derivative of the trophectoderm and morula grade. This resulted in ten classes of algorithm scores, 1-10, giving a live birth rate from 2% to 46%, irrespective of patient variables, for chance of live birth.
Limitations, reasons for caution: Successful application of the algorithm is reliant on stringent quality assurance for maintenance of accurate annotation and grading, and may not be transferable between laboratories with different SOPs.
Wider implications of the findings: The addition of a trophectoderm and morula grade in combination with morphokinetic parameters, increases the predictive value of the algorithm in relation to live birth outcome. Using proxy values allows maximization of data for model generation, and allows the model to be applied when missing values are present.
Trial registration number: not applicable

P-165 Using Artificial Intelligence to Classify Embryo Shape: An International Perspective

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Study question: Is a pre-trained machine learning algorithm able to accurately detect cellular arrangement in 4-cell embryos from a different continent?
Summary answer: Artificial Intelligence (AI) analysis of 4-cell embryo classification is transferable across clinics globally with 79% accuracy.
What is known already: Previous studies observing four-cell human embryo configurations have demonstrated that non-tetrahedral embryos (embryos in which cells make contact with fewer than 3 other cells) are associated with compromised blastulation and implantation potential. Previous research by this study group has indicated the efficacy of AI models in classification of tetrahedral and non-tetrahedral embryos with 87% accuracy, with a database comprising 2 clinics both from the same country (Brazil). This study aims to evaluate the transferability and robustness of this model on blind test data from a different country (France).
Study design, size, duration: The study was a retrospective cohort analysis in which 909 4-cell embryo images ("tetrahedral", n = 749; "non-tetrahedral", n = 160) were collected from 3 clinics (2 Brazilian, 1 French). All embryos were captured at the central focal plane using Embryoscope™ time-lapse incubators. The training data consisted solely of embryo images captured in Brazil (586 tetrahedral; 87 non-tetrahedral) and the test data consisted exclusively of embryo images captured in France (163 tetrahedral; 72 non-tetrahedral).
Participants/materials, setting, methods: The embryo images were labelled as either "tetrahedral" or "non-tetrahedral" at their respective clinics. Annotations were then validated by three operators. A ResNet-50 neural network model pretrained on ImageNet was fine-tuned on the training dataset to predict the correct annotation for each image. We used the cross entropy loss function and the RMSprop optimiser (lr = 1e-5). Simple data augmentations (flips and rotations) were used during the training process to help counteract class imbalances.

Main results and the role of chance: Our model was capable of classifying embryos in the blind French test set with 79% accuracy when trained with the Brazilian data. The model had sensitivity of 91% and 51% for tetrahedral and non-tetrahedral embryos respectively; precision was 81% and 73%; F1 score was 86% and 60%; and AUC was 0.61 and 0.64. This represents a 10% decrease in accuracy compared to when the model both trained and tested on different data from the same clinics.
Limitations, reasons for caution: Although strict inclusion and exclusion criteria were used, inter-operator variability may affect the pre-processing stage of the algorithm. Moreover, as only one focal plane was used, ambiguous cases were interpoloated and further annotated. Analysing embryos at multiple focal planes may prove crucial in improving the accuracy of the model.
Wider implications of the findings: Though the use of machine learning models in the analysis of embryo imagery has grown in recent years, there has been concern over their robustness and transferability. While previous results have demonstrated the utility of locally-trained models, our results highlight the potential for models to be implemented across different clinics.
Trial registration number: Not Applicable

P-166 Machine learning for automated cell segmentation in embryos

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Study question: Is it possible to automate the process of detecting individual blastomeres within a 4-cell embryo?
Summary answer: Deep learning models are capable of identifying individual cells in single focal plane images of 4-cell embryos.
What is known already: As individual blastomeres within a 4-cell embryo maintain totipotency, their intercellular junctions are critical in maintaining and directing communication. These junctions are determined by the zygote's cleavage patterns, and can affect the overall embryo 'shape', which can be described as either 'tetrahedral' or 'planar'. Planar embryos carry significantly worse outcomes both in the short and long term, such as more compromised blastulation, clinical pregnancy and live birth rates. Therefore, more accurate identification of cell borders at the 4-cell stage may contribute to improved classification of cell shape and embryo visualisation.
Study design, size, duration: This was a retrospective cohort analysis of 222 single focal plane images from 3 clinics. Each image captured an embryo at the 4-cell stage and was taken using the Embryoscope™ time-lapse incubator at the central focal plane. Images from two of the clinics were split into training (n=161) and validation (n=17) sets. Images from the third clinic formed a blind testing set (n=44).
Participants/materials, setting, methods: Ground truth masks were manually created by two human operators using the VGG Image Annotator software. A MaskRCNN neural network model with a pre-trained ResNet-50 backbone was trained to segment individual blastomeres from training images. Data augmentation (flips, rotations, Gaussian noise, cropping, brightness changes and optical distortion) were used during the training process. The model's performance was evaluated using the IoU metric (a measure of overlap between model-predicted and human-annotated masks).
Main results and the role of chance: The model was evaluated on a blind test set of 44 images. The model had a mean IoU of 0.92 for individual cells (Standard Deviation (SD) = 0.05) with precision and sensitivity of 0.95 and 0.97 respectively.

The mean IoU for the entire embryo (in relation to all 4 blastomeres combined) was 0.92 (SD 0.02). Furthermore, the model was able to count the number of cells in the images with 70% accuracy, and deviating by no more than 1 cell in each error. The nature of these errors can be broken down into the detection of fragmentation as a cell (2 cases); the detection of two cells as one (1 case); the cell being directly under another cell (4 cases); and duplicate detection of the same cell (6 cases). This last issue could be resolved by rejecting detections with significant overlap.

Our results demonstrate that our model can be used across different clinics.
Limitations, reasons for caution: Inaccuracies in segmentation and cell counting sometimes occurred when a cell's borders were unclear or obscured (e.g. in a different focal plane). The inclusion of multiple focal planes will be key for improving performance. Moreover, as only one focal plane was used, ambiguous cases were annotated with a 'best guess'.
Wider implications of the findings: The creation of a model capable of detecting individual cells would be highly beneficial in the IVF industry. Aside from automating laborious processes for embryologists, it may also prove a useful tool for future research such as in identifying intercellular contact points or rendering three-dimensional embryo visualisations.
Trial registration number: N/A

P-167 Assuring quality in embryology decision making: blastocyst grading agreement assessed via a smartphone application

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Study question: Given the subjectivity of blastocyst grading and the challenge of performing routine competency assessments, how consistently do embryologists grade blastocysts when using an easily accessible phone application?
Summary answer: Grading agreement was fair to moderate for inner cell mass (ICM) and trophectoderm (TE), evidence that a mobile application can be used for quality assurance.
What is known already: Embryologists routinely perform external quality assessments (EQA), though the utility of EQA for quality improvement is limited, and more active, user-friendly tools are needed to improve quality assurance in embryology. Blastocyst grading is one of the most important and subjective tasks in clinical embryology, important for both blastocyst ranking and decision to freeze. Inter-user agreement is only fair for ICM (kappa 0.349) and TE grade (kappa 0.397; Storr et al., 2017). Fair agreement has also been reported for decision to freeze for a cohort of blastocysts that exhibited borderline morphology (kappa 0.301; Hammond et al., 2020).
Study design, size, duration: A prospective study of blastocyst grading consistency using ARTCompass, a mobile phone application designed to assess clinical decision making of laboratory staff for andrology and embryology competency. Two assessments, each with 100 images of expanded blastocysts in three planes, were performed by 42 embryologists from 9 clinics in 2 countries between April to July 2020. Survey 1 assessed ICM grading and survey 2 assessed TE grading using the same set of images for consistency.
Participants/materials, setting, methods: Blastocysts were of proportionally mixed grades (ranging from grade A to X) using a modified Gardner system that included "X" for non-viable ICM/TE. Embryologists were advised to complete the tests individually in one sitting. The Fleiss kappa coefficient (k) measured inter-rater agreement among embryologists when assigning blastocyst grade. Kappa value interpretation is as follows <0.20: poor; 0.21–0.40: fair; 0.41–0.60: moderate; 0.61–0.80: good and 0.81–1.00: very good.
Main results and the role of chance: Overall, agreement for ICM and TE grades was moderate among embryologists (kappa 0.47, 0.52, respectively). ICM grade B and C had the lowest agreement (0.37, 0.39), while ICM grade X (no, or degenerate ICM), and TE grade A showed the highest agreement (0.68, 0.62). These results illustrate that embryologists had difficultly classifying ICM grade when it was of moderate to poor quality (grade b or c), likely due to subjectivity in grading size and compaction level, but were good at classifying ICM grade when there was no apparent ICM (grade x). For TE grade, embryologists consistently identified a top-quality TE (grade a), which is reassuring as TE grade is the primary morphological feature used for blastocyst ranking. In general, this QA platform offers ease of use and shows agreement values for

ICM and TE that are similar to other studies, suggesting that blastocyst grading with a mobile phone application is a viable option for quality assurance.
Limitations, reasons for caution: Only blastocyst grading was assessed, therefore additional competency assessments using a mobile device should be assessed for accuracy. Further studies are needed to determine if mobile applications can improve competency.
Wider implications of the findings: Ease of use by 42 embryologists indicates mobile applications may provide a user-friendly and accessible platform for QA. Since effective and efficient assessment of competency and KPIs is an ongoing challenge for laboratories, a mobile application is a novel and effective tool to monitor QA parameters in the IVF laboratory
Trial registration number: not applicable

P-168 RCT comparing the effect of Continuous (Single Step) embryo culture system versus a Sequential embryo culture system on the outcome of IVF/ICSI cycles

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Study question: Is the outcome of IVF/ICSI cycles done with continuous (single step) embryo culture system different from that with sequential embryo culture system ?
Summary answer: Yes the outcome of IVF / ICSI cycles done with continuous (single step) embryo-culture system is better than that with sequential embryo-culture system .
What is known already: Embryo culture media are important factors in IVF, which can significantly influence the clinical outcome of IVF/ICSI cycles. However it is not clear which formulation is most optimal and whether sequential or continuous media (single step) should be favored. Sequential media complies with embryo demands based on developmental stage , taking into account metabolic changes embryos undergo in-vivo, while moving from the oviduct to the uterus. The embryos in the early cleavage stage prefer to use pyruvate to produce energy, whereas once development nears the blastocyst stage , the embryos start using glucose in the process of glycolysis .
Study design, size, duration: A prospective RCT was carried out at our centre between 2018-2019 and IVF-ICSI patients meeting inclusion criteria (at least six normal MII - Oocytes) were included in this study. The aim of study was to compare blastocyst formation rates after embryo-culture in two different culture media systems. 436 metaphase II Oocytes from 62 women were randomly and equally divided to be fertilized and cultured to the blastocyst stage in either sequential media or single-step media.
Participants/materials, setting, methods: In this prospective trial with sibling oocytes, 436 metaphase II oocytes from 62 women were randomly and equally divided to be fertilized and cultured to the blastocyst stage in either sequential media (n = 218 MII oocytes) or a single medium (n = 218 MII oocytes). In both groups, embryos were cultured in an interrupted fashion with media changes on day 3. Embryo transfer was performed on day 5.
Main results and the role of chance: Blastocyst formation rates on day 5 were significantly higher following culture in single step media 60.55 % (132 / 218) as compared to sequential media 34.86% (76 / 218) . The percentage of good quality blastocysts was also significantly higher in single step media. In conclusion, culture in single step media was associated with higher blastocyst formation rates compared to sequential media , suggesting that the single medium may provide better support to the developing embryo. The proportion of poor quality embryos was significantly higher in the sequential media group. Results indicate that embryo culture in continuous media could be as efficient as embryo culture in sequential media. A significant difference observed was the proportion of poor quality embryos on day 5 , which was significantly higher when the embryos were cultured in sequential media. Our results suggest that the type of embryo culture media can influence the quality of embryos both at the cleavage stage and blastocyst stage. The use of continuous embryo culture media does not seem to cause an adverse effect; in fact, their use can lower the workload in busy IVF labs and lower the stress that embryos are exposed to during handling.
Limitations, reasons for caution: Although single-step-medium for extended culture has practical advantages and blastocyst formation rates appear to be higher, there is insufficient evidence to recommend either sequential or single-step media as being superior for the embryo-culture to days 5/6. Further studies comparing these two media systems in well-designed trials should be performed.
Wider implications of the findings: When employing sequential media for embryo culture , it is necessary to transfer the embryos from one medium to another (cleavage stage medium to blastocyst stage medium) which increases stress related embryo damage . Therefore, single-step media is beneficial as the embryos can develop undisturbed till blastocyst stage.
Trial registration number: not applicable

P-169 Does increasing the time interval between Oocyte-Retrieval and Oocyte-Denudation improve the results in ICSI cycles ?

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Study question: What should be the optimal time interval which elapses between oocyte retrieval and denudation followed by ICSI , for optimal results in ART cycles ?
Summary answer: Our study suggests that an optimum interval between oocyte retrieval and oocyte denudation followed by ICSI, leads to better results in ART cycles.
What is known already: It is widely accepted that the best timing for OPU is 34-39 hours after ovulation trigger. Some studies suggest that preincubation time before ICSI can be beneficial when it comes to fertilization and pregnancy rates while late ICSI (fertilization) may have negative results due to oocyte ageing. Other studies claim that there is no significant difference in ART results when ICSI is performed between 2-6 hours post Oocyte-Retrieval (OR) . Few studies state that 1-3 hours of COC-culture prior to denudation and oocyte injection is better as far as fertilization , embryo quality and improved oocyte cytoplasmic maturity is concerned.
Study design, size, duration: RCT of 234 ICSI cycles was carried out between 2017-2019. Patients were divided into two groups-: A- Early denudation with ICSI and B- Late denudation with ICSI.Both the groups were comparable in terms of female age, number of oocytes, day of transfer, number of embryos transferred and embryo quality. Fresh or frozen embryos were transferred , which were always derived from the same stimulation cycle. Exclusion criteria were : Severe male factor / TESA / PESA.
Participants/materials, setting, methods: 234 ICSI cycles with similar ovarian stimulation protocols were analyzed as per time range between triggering, OPU, denudation and ICSI. Patients were divided into two groups: A- Early denudation (1-2 hours after OPU) with ICSI (1-2 hours after denudation) and B- Late denudation (4-6 hours after Oocyte-Retrieval) with ICSI (1-2 hours after denudation).Primary outcomes were oocyte maturation and fertilization rates and secondary outcomes were clinical pregnancy rate and abortion rates.
Main results and the role of chance: In group B (Late denudation and ICSI), the mean fertilization rate was 67% and the Clinical Pregnancy rate was 46%. This was better than the mean fertilization rate of 56% and clinical pregnancy rate of 39% observed in group A (Early denudation and ICSI). However the difference was not statistically significant. Therefore, ideal maturation rates were observed when denudation (followed by ICSI) was delayed and done 4-6 hours after Oocyte-Retrieval. In ICSI cycles in ART , ovarian stimulation is used to induce the simultaneous growth of multiple follicles, followed by final maturation and ovulation triggering with exogenous hCG. or GnRH-Agonist or both. Generally, oocyte retrieval (OR) is performed 34 - 36h later. In addition, 2-4 hours in culture of the cumulus oocyte complexes (COC) prior to oocyte injection is believed beneficial for fertilization and embryo quality, probably due to improved oocyte cytoplasmic maturity. However, in large ART centers with high workloads, following such definite time intervals is frequently very difficult.
Limitations, reasons for caution: In large busy centers , maintaining meticulous time intervals is difficult . As our study numbers are small, larger multicentric trials are required in order to confirm our findings and to provide more robust data . This data cannot be applied to IVM, TESE / PESE and severe male-factor infertility.
Wider implications of the findings: To achieve a successful fertilization, both nuclear and cytoplasmic maturity are required. Our Study indicates that a

slight delay in denudation following Oocyte-Retrieval , will yield a higher number of good quality oocytes. A higher success rate can also be expected due to more number of embryos available for transfer.
Trial registration number: not applicable

P-170 The secretomy of embryo sex

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Study question: Does the analysis of the metabolites of the embryonic culture medium can predict the sex of the embryo?
Summary answer: The presence and quantity of some metabolites in the culture medium can predict the sex of the human embryos.
What is known already: Advances in analytical techniques for metabolomics have brought the possibility of better tools for the characterization of molecules. Embryonic metabolism can be used as a good indicator of viability, regardless of the morphology of the blastocysts, since differences were observed in the metabolic activities between the days of embryo development and in the rates of live births.
Study design, size, duration: 16 patients had their embryos biopsied between the months of January to July 2019 in a human reproduction laboratory. All cases had PGT-A indication and after the biopsy, the embryos were frozen. The culture medium samples were individually prepared for metabolites extraction according to the Bligh and Dyer protocol. Controlled ovarian stimulation and dose adjustments according to the response of each patient. The metabolomics analysis was performed by mass spectrometry.
Participants/materials, setting, methods: Follicular puncture were performed 35 hours after r-hCG. The eggs were kept in individual culture until the blastocyst stage. The blastocysts biopsy was performed (20). After the culture medium was sent to the 337 metabolites analysis by mass spectrometry. 14 molecules with the highest score on the PLS-Da was submitted to the ROC curves showing the power of metabolic analysis to predict the sex of euploid embryos. Besides, we performed the functional enrichment analysis.
Main results and the role of chance: After the genetic analysis by PGT-a, we obtain 20 euploid embryos, being 12 female embryos and 08 male embryos. Comparing the quantitative target metabolomic analysis of the 337 metabolites in the embryo culture medium, we observed the Asymmetric dimethylarginine, FAD, Malic Acid, Serotonin, increased in female embryos and Adenosine monophosphate, L-Alanine, L-Arginine, Cysteamine, DL-Dopa, Flavin Mononucleotide, Methionine sulfone, Nicotinic acid, L-Tyrosine, Uracil in male embryos. Through the ROC curve, we can verify AUC = 0.937. This result suggests that the metabolomic analysis of the culture medium is valid to be used as a complement of PGT-A to know embryo sex diagnostic. The functional enrichment analysis shows the Asymmetric dimethylarginine and Malic Sulfone metabolism as the principal function alter by female embryos.
Limitations, reasons for caution: Small number of samples
Wider implications of the findings: Further studies are needed to validate these findings for the diagnostic of sex embryos
Trial registration number: N/A

P-171 Automated oocyte and zygote denudation using a novel microfluidic device supervised by a computer vision algorithm

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Study question: Is it possible to remove cumulus cells using a 16-well microfluidic device with automated flows to facilitate vitrification, ICSI, NI-PGT or non-invasive metabolomics analysis?
Summary answer: The designed automated system and protocol efficiently denude 16 samples simultaneously with a x10 lower shear stress than the manual process and without human intervention.
What is known already: Most processes involved in IVF such as insemination, washing, denudation, embryo culture and selection are still manually performed, labor-intensive and require highly skilled professionals. This leads to a significant variability in the clinical outcomes achieved by different embryologists and labs. The automation of these processes is a promising approach to reduce costs and improve the accessibility to assisted reproductive therapies. Although a simple procedure, standardization of cumulus oocyte complex (COCs) and zygotes denudation is key to facilitate ICSI, vitrification and to avoid DNA contamination for NI-embryo testing (PGT or metabolomics), while avoiding damage to the oocyte by excessive shear stress.
Study design, size, duration: A total of 160 cow COCs were used due to their size similarity with human COCs. Half were denuded 16-20 hours post-insemination and half pre-insemination for 5-10 minutes. COCs were classified as partially denuded if fertilization assessment, ICSI or vitrification was possible, and completely denuded if no cumulus cells remained. COCs controls were manually denuded (Stripper® capillary 145µm ID) to compare shear stress between procedures. This study was conducted during 2020 – 2021.
Participants/materials, setting, methods: We developed a customized microfluidic biochip that exerts a particular fluid motion while avoiding egg entrapment within microfluidic channels. The denudation efficacy was established by subjectively scoring images of bovine oocytes after generating a continuous "Push & Pull" fluid motion inside the biochip wells. A Computer Vision model was developed in parallel in order to optically assess denudation completion. The model used was a Pytorch implementation of Faster-RCNN with ImageNet pretrained weights
Main results and the role of chance: 96 bovine COCs were microfuidically handled post insemination achieving complete (56/96) or partial (40/96) removal of the cumulus cells on day 1, while for day 3 double denudation group, 89/96 (92.7%) were completely denuded while the rest remained partially denuded. In comparison, 80/80 (100%) of manually denuded cow COCs, achieved complete denudation (50% post-insemination group and 50% pre-insemination group). In addition, 48/64 (75%) cow COCs treated pre-insemination were partially denuded, enough to carry out ICSI after 5-10 min of treatment. The results here obtained indicate that media needs to flow through the device at a rate that can generate enough shear to strip off the cumulus-corona cells while avoiding emptying of the reservoirs containing the fertilization or culture medium. The shear stress of our design was calculated to be smaller than 4.4 Pa, about ten times lower than the one applied by the manual process (~44Pa). The deep learning algorithm was tested on 20 unseen human oocytes on day 1, with 10 true positives 9 true negatives, and 1 false negative (95% accuracy).
Limitations, reasons for caution: The success of the denudation procedure was dependent on the design of the biochip wells and the microfluidic protocol used. The accuracy of our findings is still limited because of the difficulty in manufacturing prototype biochips.
Wider implications of the findings: Complete denudation is key to avoid DNA contamination for NI-PGT or metabolomics analysis, while avoiding damage to the oocyte by excessive shear stress. Our device, which has the potential of scaling up and treat each oocyte individually, can improve automation and increase efficiency of current ART procedures
Trial registration number: NA

P-172 Data-independent acquisition-proteomics of human embryo-spent medium and identification of potential embryo biomarkers

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Study question: Can human embryo-derived protein(s) serve as viability biomarkers to predict pregnancy outcome, post embryo transfer?
Summary answer: The human embryo-spent medium proteome, using data-independent acquisition (DIA) approach, could identify novel biomarkers for use in elective embryo transfer.
What is known already: Morphological assessment is used for elective embryo transfer. To improve IVF outcomes and to avoid multiple gestations, embryo-viability assessment is required toward single embryo transfer. Embryo proteomics could provide a non-invasive approach to assess embryo viability. With the advent of DIA mode proteomics, a robust proteome of E-SM could be determined.
Study design, size, duration: This was a retrospective study performed between May and December, 2020 using ten E-SMs obtained from ten individual transferable-quality embryos. Frozen E-SMs, following post-thaw, were subjected to LC-MS-MS analysis. Identified proteome profiles were being potentially correlated to embryo quality scores and pregnancy outcomes in terms of live births.
Participants/materials, setting, methods: The E-SMs were processed for proteomic analysis and subjected to reduction, alkylation and trypsin digestion. Trypsin digested samples were desalted followed by LC-MS/MS using DIA method. Obtained results were searched against human peptide spectral library using Skyline. Differentially expressed proteins were identified by MSStat. Individual peptide peak area under the curve was normalized and analyzed using Student t-test. Fold change was calculated to identify differentially regulated proteins in blank and E-SM samples.
Main results and the role of chance: Using a high-resolution mass spectrometer and high throughput DIA method, we identified 5,502 peptides corresponding to 3,396 proteins from blank and E-SM samples, derived from five non-transferred embryos. We observed that 516 proteins were specific to E-SMs *vies-a-vies* those of embryo-free blank medium. Statistical analysis showed that 25 proteins were significantly present E-SMs *vs.* blank. Interestingly, we observed that 16 proteins were down regulated and 9 were up regulated in E-SMs *vs.* blank medium. Furthermore, E-SMs, from transferred embryos, contained 2,467 peptides corresponding to 1,741 proteins; of these, 1, 689 proteins were specific to E-SMs with 60 (58 down regulated and 2 up regulated) of them being significantly expressed in E-SMs *vis-à-vis* embryo-free blank medium. Considering the available met analysis published data, our study is the first to use DIA acquisition for high-throughput analysis of human embryo proteome and identification of biomarkers of embryo viability and for possible prediction of pregnancy outcome.
Limitations, reasons for caution: Proteins, other than HAS, detected in the blank medium could be because of non-purified HAS or undeclared proteins and DIA approach used. A large cohort study and meta-analysis using DIA mode are required to establish the embryo-proteome having predictive potential for embryo biological viability.
Wider implications of the findings: For the first time, using DIA mode, a global embryo proteome assessment could be made, establishing a novel embryo viability biomarkers. This, along with the morphological analysis, could be practiced for selection of transferable quality embryo(s)
Trial registration number: Not Applicable

P-173 Predicting implantation: comparative performance of two embryo selection algorithms and conventional morphological grading after frozen blastocyst transfer

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Study question: What is the predictive performance of iDAScore® compared with KIDScore™ D5 and conventional morphology grading for implantation after frozen blastocyst transfer?
Summary answer: iDAScore®, KIDScore™ D5 models and conventional morphology grading are all significantly associated with implantation after frozen blastocyst transfer. However, their predictive performance remains fair.
What is known already: Embryo selection algorithms (ESA) have been used in conjunction with conventional morphology grading (CMG) to rank embryo quality in an attempt to optimise embryo selection prior to transfer. Traditionally, ESA, such as the KIDScore™ D5 prediction model, have been based on predetermined morphokinetic parameters which vary according to the specific ESA used. Recently, algorithms based on artificial intelligence (AI) deep learning such as the iDAScore®, have been developed using raw time lapse image data of embryos with known implantation outcome. Embryo scores generated by both traditional and AI based ESA have been positively correlated with implantation potential in retrospective studies.
Study design, size, duration: This retrospective single centre study included data from 157 frozen embryo transfers carried out between January 2020 and January 2021 with embryos cultured in EmbryoScope+ time-lapse incubator. Embryos were selected for transfer using CMG. iDAScore® and KIDScore™ D5 were generated retrospectively for each embryo transferred. Sensitivity and specificity for implantation in addition to concordance was determined for all three embryo scoring systems and compared.
Participants/materials, setting, methods: Statistical analysis was performed with SPSS software. ROC curve analysis was performed to investigate the predictive performance of the three embryo selection methods for implantation. Chi-Square test was used to determine if there was a significant association between iDAScore®, KIDScore™ D5 and CMG with implantation. Spearman's correlation tested for correlation between iDAScore®, KIDScore™ D5 and CMG.
Main results and the role of chance: A statistically significant but limited predictive power was observed between iDAScore®, KIDScore™ D5 and CMG methods of embryo selection and implantation rate (AUC=0.675, 95% CI 0.59, 0.76; AUC=0.683, 95% CI 0.60, 0.77 and AUC=0.638, 95% CI 0.55, 0.73) respectively. Unsurprisingly, higher values for each of iDAScore®, KIDScore™ D5 and CMG were significantly associated with higher implantation (p=0.002, p<0.001, p=0.003) respectively. Interestingly, there was a significantly moderate correlation between iDAScore® and CMG rs (155)=0.581, p<0.001, while a significantly strong correlation between KIDScore™ D5 and CMG rs (155)=0.775, p<0.001 as well as between iDAScore® and KIDScore™ D5 rs (155)=0.799, p<0.001.
Limitations, reasons for caution: This is a retrospective single centre study with limited sample size, hence the results may be interpreted with caution. Further analysis involving a larger patient cohort must be carried out prior to implementing iDAScore® or KIDScore™ D5 as primary embryo selection methods.
Wider implications of the findings: The findings of this study support the potential application of iDAScore® and KIDScore™ D5 prediction models in automatic embryo selection, thereby minimising operator variability and time taken for embryo grading.
Trial registration number: Not Applicable

P-174 Globulin-rich protein supplements improve blastulation efficiency in culture and promote implantation in vitro

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Study question: Can globulin-rich compared to albumin (HSA) supplements improve blastulation and support embryo development towards post implantation?
Summary answer: Yes, globulin supplements with clinical-grade quality increase blastulation efficiency by 20% (50% in older mothers) and support the transition of embryos towards post-implantation development.
What is known already: During embryonic development at the morula stage there is a metabolic transition towards glycolysis as demand from outsourced energy increases. Therefore as cleavage proceeds, the demand for nutrients in the embryo increases accordingly.

With few exceptions, HSA from human plasma or recombinant origin has been the main an only protein supplement used in almost all IVF-procedures. Globulin rich supplements are available but their use is not widespread and little is known about their efficiency in post-implantation development.
Study design, size, duration: We have cultured more than 600 mouse embryos in continuous media containing a protein supplement#1 (PS#1), from 1-cell up to blastocyst stage. At blastocyst stage embryos were replaced into fresh media containing protein supplement#2 (PS#2). The embryos were allowed to hatch naturally and then transferred into a proprietary matrix for further development and implantation for an additional 48h. Participants/materials,

setting, methods: The blastulation rate, measured for HSA-supplemented embryo cohort was compared with embryos cultured in PS#1. Hatching efficiency was reported for embryos cultured in transfer media including PS#2. Once embedded in the matrix, advanced label-free imaging techniques and custom algorithms to measure matrix implantation strength were used. Key molecular markers (i.e. OCT4, CDX2) for correct post-implantation lineage patterning were documented by conventional 3D confocal immunofluorescence imaging.

Main results and the role of chance: Embryos supplemented with PS#1 reached blastocyst with overall 21% higher efficiency than embryos supplemented by HSA. When separated by age cohorts, embryos obtained from older females (ex-colony breeders, >14 weeks old) reached blastocyst stage with 55% higher efficiency than the same type of embryos cultured in the presence of HSA. Embryos obtained from females at optimal reproductive age reached blastocyst stage 10% more efficiently under PS#1 supplementation than with HSA. Hatching efficiency was 45% higher for embryos cultured with PS#2 than embryos supplemented with HSA. For every variable tested (e.g. % of arrested or degenerated embryos) or condition implemented (e.g. mouse basal media, human basal media from different brands, etc.) PS#1 and PS#2 outperformed, without exception, the supplementation with HSA.

When embedded in the implantation matrix, the embryos cultured with PS#1 (cleavage) and transferred to PS#2 at blastocyst stage showed a remarkable implantation ability as measured by trophoblast outgrowth and matrix deformations. The embryos in PS#2 medium exerted stronger force into the matrix and also survived longer times than the embryos in HSA. PS#2 supported the transition of blastocyst towards post-implantation stages of development showing the correct lineage patterning of embryonic and extraembryonic molecular markers, including Oct4, CDx2, EOMES or GATA4.

Limitations, reasons for caution: This is a study based on an animal model. These observations need to be confirmed by ongoing experiments with human embryos.

Wider implications of the findings: This work constitutes a proof-of-concept for the use of globulin-rich supplements as higher performance substitute of albumin in the culture of IVF embryos, both as (i) a standard protein source for culture media and (ii) as a supplement for transfer media to capacitate the embryo for implantation.

Trial registration number: not applicable

P-175 Possible role of NAD+ metabolism in oocyte aging: insights from gene expression profiles in reproductive aged oocytes

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Study question: Does reproductive aging alter the expression of genes involved in NAD+ metabolism in the mammalian oocyte?

Summary answer: We found that aging alters the expression of thirty genes encoding for NAD+-producing and NAD+-consuming enzymes pathway in mouse MII oocytes.

What is known already: NAD, a multifunctional metabolite in living cells, is known to convert between its oxidized NAD+ and reduced NADH forms during nutrients breakdown; the intracellular NAD+/NADH redox state reflects cell ability in generating ATP energy. NAD+ is utilized by proteins that control gene expression, DNA repair, apoptosis, mitochondrial biogenesis (i.e. sirtuins). Raising NAD+ by inducing its biosynthesis leads to sirtuins activation, so directly linking the cellular redox state with signalling events. The NAD+ pool is set by a critical balance between NAD+ biosynthetic and NAD+ consuming pathways. NAD(P)H levels declined in aged oocytes and NAD+ precursors seem to counteract ovarian aging.

Study design, size, duration: Pools of 25 oocytes at MII stage were obtained from young (4–8 weeks) and reproductively aged (48–52 weeks) CD-1 mice and processed for the analysis of 41 genes participating in NAD+ biosynthetic and NAD+ consuming pathways NAD+ pathways. Each experiment was performed three times and data were subjected to bioinformatic analysis to unravel potential age-related effects on NAD metabolism.

Participants/materials, setting, methods: Mice were superovulated by intraperitoneal injection of PMSG followed by hCG 48h apart. MII oocytes were isolated by 0.3 mg/ml hyaluronidase. RNA was obtained from each sample by Arcturus PicoPure Kit, and reverse transcribed. Each cDNA was analysed in triplicate by employing a NAD Metabolism H41 Predesigned panel for use with SYBR® Green, containing 41 genes of the NAD pathway, 2 housekeeping genes and 6 control probes. Raw data were analysed by DataAssist software.

Main results and the role of chance: The comparison between aged and young oocytes were focused on genes showing an absolute fold change (FC) <0.7 or > 1.4, a present call in at least the 50% of experiments and a p-Value <0.05 (ANOVA). Excluding transcripts showing a concordant value < 80%, n.30 differentially expressed genes (DEGs) were found: n.26 transcripts down-expressed and n.4 genes up-regulated. Data obtained by Ingenuity Pathways Analysis (IPA) software (Ingenuity Systems) provide evidence that NAD+ biosynthesis in aged oocytes is severely compromised.

Limitations, reasons for caution: Our results on animal model must be taken with caution. Validation of NAD+ precursor or activators of NAD+ biosynthesis in vivo administration is required.

Wider implications of the findings: Present results demonstrate that aging affect oocyte genes involved in the regulation of NAD+ availability and supports the hypothesis that modulation of NAD+ metabolism may be an "anti-aging" strategy. Overall, these data laid the foundation for a central role of NAD+ metabolism in the maintenance of oocyte competenc

Trial registration number: not applicable

P-176 Effects of high non-esterified fatty acids exclusively during bovine in vitro fertilization on cell lineage allocation of blastocysts

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Study question: Are there effects of high non-esterified fatty acids exclusively during bovine in vitro fertilization on cell lineage allocation of blastocysts?

Summary answer: Under the conditions of the present study, high exposure to NEFA during bovine IVF significantly decreases embryo production and alters cell allocation of resultant blastocysts.

What is known already: Cattle models have shown that a high exposure to non-esterified fatty acids (NEFA) such as steric acid (SA), palmitic acid (PA]) and oleic acid (OA) during in vitro oocyte maturation and embryo development can disrupt both embryo formation and quality. However, the fertilization process per se have been less studied, which is needed to identify developmental stages were potential therapies could be develop to ameliorate NEFA toxicity during the periconceptional period

Study design, size, duration: Day-8 blastocysts were immunostained for CDX2, a transcription factor involved in trophectoderm differentiation, to examine cell allocation of blastocysts derived from oocytes fertilised under high NEFA levels. VF (19 h) was carried with different NEFA levels(4 replicates) representing physiological (Control-1[C1], 28μM SA, 23μM PA, 21μM OA) and pathophysiological (NEFA, 280μM SA, 230μM PA, 210μM OA) relevant concentrations. A second control (C2) group contained solvent. Blastocysts (C1; n=14, C2; n=12, NEFA; n=8)

Participants/materials, setting, methods: All blastocysts were examined by confocal microscopy and cell counting was done with the Imaris software. Data were analysed by ANOVA (mean±SEM) with percentage data arcsine transformed before analysis.

Main results and the role of chance: Blastocyst formation was decreased by high NEFA levels (C1=25.6±2.7%, C2=26.0±2.3%, NEFA=9.4±0.4%, P<0.001) which was associated with a decreased cleavage rate (C1=70.1±6.5%, C2=71.5±3.1%, NEFA=42.5±4.1%, P=0.006) and an increase in embryo degeneration (C1=47.6±3.5%, C2=47.7±5.8%, NEFA=63.0±4.9%, P=0.05). A lower total cell (TC) the number was observed in high NEFA-derived blastocyst (C1=125.2±6.6, C2=132.3±8.4, NEFA=67.3±5.6, P<0.001) associated with a

low cell number in both the trophectoderm (CDX2 positive cells, C1=90.2±5.9, C2=96.7±6.4, NEFA=41.3±4.1, P<0.001) and the inner cell mass (ICM, C1=35.0±2.4, C2=35.7±3.5, NEFA=26.0±2.2, P<0.001). Furthermore, high NEFA-derived blastocyst showed an increased allocation of cells towards the ICM (ICM/TC proportion, C1=28.2±1.7%, C2=26.9±1.8%, NEFA=39.1±2.2%, P<0.001)

Limitations, reasons for caution: It will be better if the number of blastocysts reached increases.

Wider implications of the findings: such research can be widely applied to the human model due to the similarities between both specie.

Trial registration number: Royal Embassy of Saudi Arabia Cultural Bureau

P-177 Seminal transferrin levels are linked to embryo utilization rates: a prospective observational study

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Study question: What is the impact of seminal transferrin level on intracytoplasmic sperm injection (ICSI) cycle outcomes?

Summary answer: Seminal transferrin levels were positively correlated with embryo utilization rates.

What is known already: Transferrin, a glycoprotein involved in iron transport throughout the body, is produced by various organs such as liver, central nervous system, and testicules. Particularly, Sertoli cells synthesize transferrin to regulate their phagocytotic activity and to ensure iron transportation to germ cells across the blood-testis barrier. In this context, studies have demonstrated the importance of transferrin and iron in spermatogenesis by detecting low levels of seminal transferrin in oligozoospermic men. However, the effects of low seminal transferrin levels on the functionality of spermatozoa and on their ability to maintain embryo development were neither fully investigated nor well understood.

Study design, size, duration: A prospective study was conducted at Al Hadi IVF center, Lebanon, from July 2019 until May 2020. It included 60 infertile couples who have undergone ICSI cycles. Couples were categorized into two groups based on basic semen parameters assessed according to the World Health Organization recommendations 2010: normozoospermia group (n=30 couples) and non-normozoospermia group (n= 30 couples). In this study, non-normozoospermia was defined as the presence of at least one abnormality in basic semen parameters.

Participants/materials, setting, methods: In this prospective study, the inclusion criteria were: couples where women had 38 years old or less at the time of ICSI, using fresh gametes, and using ejaculated semen. Moreover, women with premature ovarian failure, obese women, and ICSI cycles with embryo biopsy were excluded. Seminal levels of transferrin and iron were measured using Cobas 400 plus (Roche Diagnostics). Fertilization rates, embryo utilization rates, and live birth rates were calculated.

Main results and the role of chance: There were no statistically significant differences in the characteristics of the study population (maternal age, paternal age, maternal body mass index (BMI), paternal BMI, maternal and paternal tobacco intake, maternal and paternal alcohol consumption, number of collected oocytes and number of embryos transferred) (p>0.05) between normozoospermia and non-normozoospermia groups. A statistically significant difference was detected in seminal transferrin levels (mg/dl) (2(0-23) *vs.* 1(0-6), p<0.02) between the two groups. Interestingly, these transferrin levels were positively correlated with sperm concentration (R = 0.29; p=0.03). In parallel, no statistically significant difference was observed between groups concerning seminal iron levels (µg/dl) (51(21.1-100.8) *vs.* 48.6 (11-85.7); p=0.33). Furthermore, there was no statistically significant difference in the fertilization rates (%) between the two groups (74.2 *vs.* 74.8; p=0.95). Of particular interest, a statistically significant positive correlation was detected between seminal transferrin levels and embryo utilization rates (R=0.33; p=0.013). In contrast, the live birth rates (%) were not different between groups (54.54 *vs.* 58.3; p=0.77) and were not correlated with seminal transferrin levels (R=-0.3; p=0.16).

Limitations, reasons for caution: A prospective study with a larger sample size is required to confirm the effects of seminal transferrin levels on assisted reproductive technology outcomes.

Wider implications of the findings: Deciphering the molecular mechanism by which transferrin level may influence spermatozoa quality and subsequently embryo development will be of paramount importance. Moreover, understanding the reasons behind the altered seminal transferrin level and testing several strategies to restore it, may enhance spermatozoa quality and increase pregnancy chances.

Trial registration number: Not applicable

P-178 Mural granulosa cells of the human follicles indirectly show death molecular signals not depending on different ovarian stimulation protocols

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Study question: Could the expression of the anti-apoptotic molecules AKT, p-Akt and ERK1/2 in Mural Granulosa Cells (MGC) be considered as marker of oocyte quality?

Summary answer: MGCs activate cell death pathways in analyzed follicles and it is not influenced by different stimulation protocols and it is not correlated to oocyte competence.

What is known already: It has been previously demonstrated that apoptosis rate of mural granulosa or cumulus cells (CC) were correlated with follicular oocyte number, age, embryo numbers in IVF/ICSI and also clinical pregnancy. Moreover, our previous data demonstrated that in selected patients, who received recombinant LH associated with recombinant FSH (rFSH), the DNA fragmentation in cumulus cells was significantly lower and pregnancy rate was higher, compared to patients treated with rFSH alone. However, to date little is known about the differences between MGC and CC regarding death/survival pathways and whether the two cell types respond in the same way.

Study design, size, duration: Molecular study on MGCs to investigate the role of the surviving/apoptotic molecules AKT, p-Akt and ERK1/2 and their relationship with the administration of exogenous r-LH combined with r-FSH administration in ovarian stimulation comparing with r-FSH alone. We analyzed also the oocyte competence, for each follicle, according to the embryo development during *in vitro* culture and the pregnancy outcome. We included fifty-three normo-responder women undergoing ICSI in two years.

Participants/materials, setting, methods: Patients were divided into two groups: 1) 34 women were stimulated with r-FSH and used as control group, 2) 19 women were stimulated with r-FSH combined with r-LH. Mural granulosa cells isolated singularly from 255 MII oocytes of the 53 patients were used for the study. The study was conducted in public university laboratory. MGCs obtained from each single follicle were suspended in medium, without serum. For immunoreaction anti-AKT, p-AKT, ERK1/2 antibodies were used.

Main results and the role of chance: Out of 255 MII oocytes collected, 197 were fertilized and the derived embryos had the following evolution: 117 transferred, 57 vitrified and 23 arrested during *in vitro* culture. 58 oocytes were not analyzed because of failed fertilization or because of their immature condition (GV or MI).

In the MGCs isolated from the follicle of each oocyte generating an embryo, the expression AKT, pAKT and ERK1/2 was analyzed and associated with embryo quality and pregnancy outcomes.

Immunoblot analysis on granulose cells showed no statistically significant differences in protein level in MGCs isolated from oocytes that have generated transferred embryos (blastocyst at day 5 or 6) comparing with embryos who arrested during *in vitro* culture. No differences were found also in MGCs collected from the follicles derived from r-FSH ovarian stimulation compared to r-FSH+r-LH. Moreover, no difference was highlighted even between protein level and pregnancy outcomes. The results seem to demonstrate that the MGCs primarily have an endocrine function and support the growth of the follicle, and

finally follow a specific death pathway. This condition is not influenced by different ovarian stimulation protocol, in contrast with CC, and is not correlated to oocyte competence, embryo quality and clinical outcomes.
Limitations, reasons for caution: Only a limited number of patients have been observed.
Wider implications of the findings: Our current and past results suggest that the evaluation of cumulus cells and mural granulosa cells in the same follicle show different expression of molecules involved in the apoptotic pathway and therefore they cannot be used, as molecular markers, in the same way, to assess the competence of oocytes.
Trial registration number: not applicable

P-179 Timing of blastocyst observation on day 5: effect on the assessment to predict live birth, and the incorporation into a blastocyst selection model

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Study question: Does variation in day 5 observation timing confound embryo-morphology-based live birth prediction, and is it possible to develop a robust comprehensive numerical prediction model.
Summary answer: Day 5 observation timing confounds embryo-morphology-based live birth prediction. A robust comprehensive numerical prediction model can be developed after considering a number of contributing variables.
What is known already: Embryo development is a dynamic process, and therefore the widely used static observations potentially lead to biased prediction of live birth outcomes. So far, little is known in regard to potential confounding impact of day 5 assessment timing on the static-morphology-based live birth prediction. In addition, the inter-observer variation in morphology-based embryo assessment requires a more robust system to improve consistency of selection.
Study design, size, duration: This retrospective multi-center cohort study included 8866 autologous oocyte *in vitro* fertilisation treatment cycles performed at 14 associated clinics within the same network during 2012-2018. Only fresh cycles with single day 5 embryo transfers were included for analysis with all pregnancies followed up until birth. Repeat cycles of same patients were excluded to avoid clustering effect in statistical analysis.
Participants/materials, setting, methods: Dataset was randomly split into two subsets at 60:40 ratio, with one (n=5274) used for regression analysis and model development and the other (n=3592) used for model testing. Multiple logistic regression was performed to evaluate live birth predicting power of several potential contributors, expressed by odds ratio (OR) and 95% confidence interval (CI). A comprehensive prediction model was subsequently developed based on calculated weights of contributing factors, then tested via receiver operating characteristics (ROC) analysis.
Main results and the role of chance: The timings of day 5 observation of 8866 included embryos, measured by hours post insemination (HPI), distributed in a bell shape ranging from 112.0 to 120.0 h (mean±SD 115.7±1.7 h). After taking into account female age at egg collection (grouped as <30 yr, 30-34 yr, 35-39 yr, 40-44 yr, and 45 yr or older), whether or not the first egg collection, number of eggs collected, embryo developmental stage (grouped as pre-blastocyst, early blastocyst, expanding blastocyst, expanded blastocyst, and hatching/hatched blastocyst) and morphology score(A/B/C/D); multivariate logistic regression analysis showed significant association (OR 1.096, 95% CI 1.020-1.177, P=0.012) between HPI groups (112-113.9 h, 114-115.9 h, 116-117.9 , and 118-120 h) and subsequent live birth outcomes. A comprehensive numerical scoring system was developed based on the statistically significant predictors including female age (OR 1.465, 95% CI 1.364-1.574, P=0.000), embryo developmental stage (OR 1.341, 95% CI 1.244-1.445, P=0.000), morphology score (OR 1.520, 95% CI 1.392-1.661, P=0.000) and HPI (OR mentioned above); with a formula of Score=(Female_age_group/5)*1.465+(Developmental_stage/5)*1.341+(Morpho_Score/4)*1.520+(HPI_Group/4)*1.096. ROC analysis showed statistically significant predictive power of the resulting model as expressed by area under the ROC curve using both the development (0.690, 0.675-0.704, P=0.000) and testing (0.685, 0.667-0.703, P=0.000) subsets.
Limitations, reasons for caution: The retrospective design does not allow for controlling of unknown confounders. HPI was based on static observations in this study so future time-lapse study may bring more insights with more accurate observation and measurement.
Wider implications of the findings: The varying HPIs at day 5 observation were alarming as this could confound live birth prediction using embryology parameters. It is important to standardise the timing of embryo observations. The inclusion of HPI into a comprehensive numerical scoring system for live birth prediction may potentially improve its robustness
Trial registration number: not applicable

P-180 Bisphenols are present in culture media used for ART and cell culture

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Study question: Do plastic laboratory consumables and cell culture media used in human ART contain bisphenols?
Summary answer: Human embryo development media contained bisphenols close to the nanomolar concentration range while no release of bisphenols by plastic consumables was detected under routine conditions.
What is known already: The deleterious effect of the endocrine disruptor bisphenol A (BPA) on female fertility raised concerns regarding ART outcome. BPA was detected neither in media nor in the majority of plastic consumables used in ART, however it might have already been replaced by its structural analogs, including bisphenol S (BPS).
Study design, size, duration: Seventeen plastic consumables and 18 cell culture and ART media were assessed for the presence of bisphenols.
Participants/materials, setting, methods: Ten different bisphenols (bisphenol A, S, AF, AP, B, C, E, F, P, and Z) were measured using an isotopic dilution according to an on-line solid phase extraction / liquid chromatography/mass spectrometry method.
Main results and the role of chance: While all the plastic consumables of this study did contain bisphenols, none of them did release bisphenols under routine conditions. Moreover, 16 of the 18 cell culture and ART media assessed contained bisphenols, including 8 among the 10 media used in human ART. Five human ART media exhibited bisphenol concentrations higher than 0.8 nM and reached up to 3.2 nM (799 ng/L).
Limitations, reasons for caution: Further studies are required to investigate a greater number of ART media to identify less potentially harmful ones, in terms of bisphenol content.
Wider implications of the findings: As BPS has already been reported to impair oocyte quality at nanomolar concentrations, its presence in ART media, at a similar concentration range, could contribute to a decrease in the ART success rate. Thus far, there has been no regulation of these compounds in the ART context.
Trial registration number: Not applicable

P-181 Morphine regulates BMP4 growth factor and is involved in in-vitro early embryo development and PGCs formation

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Study question: To elucidate if morphine can alter embryo development.
Summary answer: Chronic morphine treatment regulates BMP4 growth factor, in terms of gene expression and H3K27me3 enrichment and promotes *in-vitro* blastocysts development and PGC formation.
What is known already: BMP4 is a member of the bone morphogenetic protein family, which acts mainly through SMAD dependent pathway, to play an important role in early embryo development. Indeed, BMP4 enhances

pluripotency in mouse embryonic stem cells (mESCs) and, specifically, is involved in blastocysts formation and primordial germ cells (PGCs) generation. Although, external morphine influence has been previously reported on the early embryo development, focus on implantation and uterus function, there is a big concern in understanding how environmental factors can cause stable epigenetic changes, which could be maintained during development and lead to health problems.

Study design, size, duration: First, OCT4-reported mESCs were chronically treated with morphine during 24h, 10-5mM. After morphine removal, mESCs were collected for RNA-seq and H3K27me3 ChIP-seq study. To elucidate the role of morphine in early embryo development, two cell- embryos stage were chronically treated with morphine for 24h and *in-vitro* cultured up to the blastocyst stage in the absence of morphine. Furthermore, after morphine treatment mESCs were differentiated to PGCs, to elucidate the role of morphine in PGC differentiation.

Participants/materials, setting, methods: Transcriptomic analyses and H3K27me3 genome wide distribution were carried out by RNA-Sequencing and Chip-Sequencing respectively. Validations were performed by RNA-RT-qPCR and Chip-RT-qPCR.

Main results and the role of chance: Dynamic transcriptional analyses identified a total of 932 differentially expressed genes (DEGs) after morphine treatment on mESCs, providing strong evidence of a transcriptional epigenetic effect induced by morphine. High-throughput screening approaches showed up *Bmp4* as one of the main morphine targets on mESCs. Morphine caused an up-regulation of *Bmp4* gene expression together with a decrease of H3K27me3 enrichment at promoter level. However, no significant differences were observed on gene expression and H3K27me3 enrichment on BMP4 signaling pathway components (such as *Smad1, Smad4, Smad5, Smad7, Prdm1* and *Prmd14*) after morphine treatment. On the other hand, the *Bmp4* gene expression was also up-regulated in *in-vitro* morphine treated blastocyst and *in-vitro* morphine treated PGCs. These results were consistent with the increase in blastocyst rate and PGC transformation rate observed after morphine chronic treatment.

Limitations, reasons for caution: To perform the *in-vitro* analysis. Further studies are needed to describe the whole signaling pathways underlying BMP4 epigenetic regulation after morphine treatment.

Wider implications of the findings: Our findings confirmed that mESCs and two-cell embryos are able to memorize morphine exposure and promote both blastocyst development and PGCs formation through potentially BMP4 epigenetic regulation. These results provide insights understanding how environmental factors can cause epigenetic changes during the embryo development, leading to alterations and producing health problems/diseases

Trial registration number: not applicable

P-182 The mechanism of mouse embryo hatching and the impact of laser drilling the zona pellucida: an RNA sequencing study

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Study question: What is the mechanism of embryo hatching? Will laser-assisted zona pellucida (ZP) drilling alter the embryonic transcriptome?

Summary answer: Hatching is an ATP-dependent process. Hatching is also associated with Rho-mediated signaling. Laser-assisted ZP drilling might cause alternation in embryo metabolism.

What is known already: Embryo hatching is a vital process for early embryo development and implantation. Animal data suggests that hatching is the result of multiple factors, such as mechanical pressure, protease activation, and the regulation of maternal secretions. However, little is known about the regulatory signaling mechanisms and the molecules involved. In addition, despite the extensive use of laser-assisted ZP drilling in the clinic, the safety profile of this technique at molecular level is very sparse. The impact of this technique on the embryonic transcriptome has not been studied systematically.

Study design, size, duration: Eighty mouse embryos were randomly divided into a laser ZP drilling group (n=40) and an untreated group (n=40). After treatment, embryos were cultured *in vitro* for two days. Then, hatching blastocyst (n=8) and pre-hatching blastocyst (n=8) from the untreated group, and the hatching blastocyst from the treatment group (n=8) were processed for RNA sequencing (RNA-seq).

Participants/materials, setting, methods: Cryopreserved 8-cell stage mouse embryos (B6C3F1 × B6D2F1) were thawed, and a laser was used to drill the embryo ZP in the treatment group. Next, the treated and untreated embryos were individually cultured *in vitro* to the E4.5 blastocyst stage. The resulting blastocysts were lysed individually and used for subsequent cDNA library preparation and RNA-seq. Following data quality control and alignment, the RNA-seq data were processed for differentially expressed gene analysis and downstream functional analysis.

Main results and the role of chance: According to the RNA-seq data, 275 differentially expressed genes (DEGs) (230 up-regulated and 45 down-regulated, adjusted $P<0.05$) were identified when comparing hatching and pre-hatching blastocysts in the control groups. Analysis suggested that the trophectoderm is the primary cell type involved in hatching, and revealed the potential molecules causing increased blastocyst hydrostatic pressure (*Aqp3* and *Cldn4*). Functional enrichment analysis suggested that ATP metabolism and protein synthesis were activated in hatching blastocysts. DEGs were found to be significantly enriched in several gene ontology terms, particularly in terms of the organization of the cytoskeleton and actin polymerisation ($P<0.0001$). Furthermore, according to QIAGEN ingenuity pathway analysis results, Rho signaling was implicated in blastocyst hatching (*Actb, Arpc2, Cfl1, Myl6, Pfn1, Rnd3, Septin9,* z-score=2.65, $P<0.0001$). Moreover, the potential role of hormones (estrogen (z-score=2.24) and prolactin (z-score=2.4)) and growth factors (AGT (z-score=2.41) and FGF2 (z-score=2.213)) were implicated in the hatching process as indicated by the upstream regulator analysis. By comparing the transcriptome between laser-treated and untreated hatching blastocysts, 47 DEGs were identified (adjusted $P<0.05$) following laser-assisted ZP drilling. These genes were enriched in metabolism-related pathways ($P<0.05$), including the lipid metabolism pathway (*Mvd, Mvk, Aacs, Gsk3a, Pik3c2a, Aldh9a1*) and the xenobiotic metabolism pathway (*Aldh18a1, Aldh9a1, Keap1,* and *Pik3c2a*).

Limitations, reasons for caution: Findings in mouse embryos may not be fully representative of human embryos. Furthermore, the mechanism of hatching revealed here might only reflect the hatching process of embryos *in vitro*. Further studies are now necessary to confirm these findings in different conditions and species to determine their clinical significance. Wider implications of the findings: Our study profiled the mouse embryo transcriptome during *in vitro* hatching, identified potential key genes and mechanisms for future study. In addition, for the first time, we revealed the impact of laser-assisted ZP drilling on the transcriptome, this may help us to assess and improve the existing technique.

Trial registration number: not applicable

P-183 Air quality oscillations inside the IVF laboratory do not affect clinical outcomes

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Study question: Do air contaminant oscillations impair in vitro fertilization clinical results?

Summary answer: Oscillations of the main air contaminants (SO2, NO, NO2, O3, CO, PM10, C6H6) inside the IVF laboratory do not impair success rates.

What is known already: Pollution is a challenge that as humans we face around the world. Given the limited number of studies that demonstrate the effect of pollution into IVF treatments, the effect that air contaminants have on *in vitro* human gametes/embryos is not clear.

IVF laboratories are designed to limit the stress that gametes and embryos suffer during culture and manipulation. Controlling temperature, humidity, light, and filtering the air is essential to have a successful IVF program. However, HEPA and active carbon filters are not enough to ensure that gametes/embryos are not exposed to contaminants, exposing them to potentially harmful gases and particles.

Study design, size, duration: Prospective study comprising treatments throughout 2019, recording levels of the main air contaminants (SO2, NO, NO2, O3, CO, PM10, C6H6) every 10 minutes inside the IVF laboratory in order to assess the effect of these pollutants. We included egg donor cycles without PGT-A.

Participants/materials, setting, methods: A total of 724 egg donation treatments were included. Using uninterrupted culture (Global, CooperSurgical) in time lapse incubators (Embryoscope, Vitrolife). A mean concentration of

every pollutant during the 6 days of every treatment was calculated. We analyzed success rates such as fertilization rates, blastocyst rates, pregnancy rates, implantation rates, miscarriage rates, and live birth rates.
Main results and the role of chance: Our results show that no contaminant affects neither fertilization rates nor good quality blastocyst rates.

The only pollutants that have an association with pregnancy rates are NO and CO (p=0.014 y p=0.021) in both the univariate and the multivariate statistical analysis. Still, this association is week and could be explained due to the large data set. When analyzing further data we do not find any association between the dose of contaminants and implantation rates, miscarriage rates nor live birth rates (p>0.01) demonstrating that oscillations in levels of these contaminants do not affect clinical results.

Our results differ with the results from a previous study where they detected an effect of SO2 and O3 when analyzing frozen embryo transfer results. This might be explained because the levels of these gases were lower in our clinic and the pregnancy and live birth rates are higher.
Limitations, reasons for caution: Although we measured the levels of the contaminants inside the IVF laboratory, we did not measure the levels inside the incubators.
Wider implications of the findings: This results show that IVF success rates are not impaired by oscillations in air quality if the laboratory does use the necessary HEPA and active-carbon air filter systems.
Trial registration number: Not applicable

P-184 Assisted hatching does not improve live birth rates, a prospective double-blinded randomized study.

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Study question: Does Assisted hatching (AH) improve success rates when applied to frozen embryo transfers?
Summary answer: AH does not improve implantation, ongoing pregnancy or live birth rates when applied to thawed embryos.
What is known already: Vitrification has been proven to be the most efficient technique to preserve human embryos. However, vitrification has some consequences for the embryos, zona pellucida (ZP) hardening being one of them. Multiple studies suggest the need to apply laser Assisted hatching or ZP thinning to thawed embryos in order to improve success rates. Still, there is not enough evidence to ensure the utility of AH, and considering the great variation in design between studies more evidence is needed.
Study design, size, duration: Study performed from October 2019 and January 2020. Disregarding embryos with natural Hatching and PGT-A. Embryos that, immediately after thawing, were completely expanded (trophectoderm in contact with ZP) were also excluded from the study. We applied a randomization to choose in which embryos we had to perform AH. Neither the gynecologist nor the embryologist performing the embryo transfer knew whether the embryo had AH performed or not.
Participants/materials, setting, methods: 353 frozen embryo transfers of one blastocist were considered for the study, 71 excluded for expansion after thawing, 65 excluded because of PGT-A, 103 in which we performed AH (AH+) and 114 without AH (AH-). In the AH+ group we performed laser-AH of 1/3 of the ZP, avoiding to damage the trophectoderm and performing the laser shots as far away to the ICM as possible. We used Chi-square testing to assess the effects of AH.
Main results and the role of chance: We assessed all relevant clinical data parameters. No statistical differences were found in egg age, maternal age, embryo quality, nor endometrial thickness between groups. Implantation and miscarriage rates were equivalent between AH+ group (40.9%; 20.5%) and AH- group (47.4%; 18.5%).

The main outcome of this study was live birth rates. No statistical differences were found between groups (AH-= 38.6%; AH+= 30.1%; p=03221) proving that making it easier to get out of the ZP does not affect success rates.

Analyzing the data from the excluded embryos we found no improvement on live birth rates when embryos were expanded just after thawing (38.0%; p=0.457). As expected, PGT-A embryos yielded higher live birth rates (52.3%; p<0,05)
Limitations, reasons for caution: Preliminary study with a small data set.
Wider implications of the findings: This study suggest that thawed embryos have the capacity to get out of the ZP regardless if AH was performed or not. Having no positive effects, AH seems to be unnecessary in this scenario.
Trial registration number: Not applicable

P-185 Overcoming a sudden COVID-19 lockdown and other similar unforeseen adverse events: Oocyte-sperm cryopreservation 4 hours after conventional in vitro insemination

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Study question: Can human oocytes be cyropreserved when they were previously inseminated while obtaining normal fertilization, embryo development and pregnancies?
Summary answer: Cryopreservation of recently inseminated human oocytes produces normal 2PN zygotes, viable embryos and ongoing pregnancies.
What is known already: Oocyte vitrification is a well-established technique in the infertility field supported by its effectiveness to freeze and thaw embryos at various stages of development. However, it is yet unknown whether cryopreservation performed in a period between conventional in vitro insemination and the appearance of pronuclei, affects any of the steps leading to fertilization, thereby arresting this process.
Study design, size, duration: Case report that includes 5 IVF cycles where cryopreservation and thawing were performed in a pre-zygote stage, 4 hours after the conventional insemination of oocytes. The rationale for this procedure was to comply with a government restriction to circulate and work in order to prevent the spread of the SARS-CoV-2 virus, unexpectedly 3 days ahead (effective on 03/19/2020). Participants/materials, setting, methods: Off-site embryologists cancelled all pending embryo transfers and returned home (2,000 km away) before the stay-home quarantine. Five couples were informed and consent was obtained on the urgent need to cryopreserve their already inseminated oocytes despite of the limited scientific information. We vitrified 28 oocyte-sperm pairs in vitrification devices containing 2 to 3 inseminated oocytes each.

Main results and the role of chance: Eight months later all non-essential activities, including non-urgent procedures in hospital facilities, were permitted again, allowing embryologists to travel and resume their postponed work. We thawed 19 of the so-called "pre-zygotes" corresponding to 4 of the 5 initial couples. Female ages were 29, 39, 38 and 37 years old. Our primary outcomes were detection of pronuclei and the occurrence of fertilization. The secondary outcome was pregnancy success. On day 1, we observed the presence of 2PN in 16 oocytes (84%). Only one patient with 2 oocytes failed to fertilize. We transferred 2 good-to-fair-grade cleaved embryos to each of the 3 patients, obtaining one pregnancy with a positive fetal heartbeat from the 39 years old patient. Two of the couples were also able to cryopreserve 2 and 1 surplus embryos on day 5, respectively.
Limitations, reasons for caution: Our strategy was used exclusively to overcome an unexpected adverse event while complying with State and County regulations on COVID-19. As a case report, a large sample size is needed to confirm and extend these results including the putative detrimental effects of arresting the fertilization process through vitrification.
Wider implications of the findings: Although cryopreservation implies to arrest of any stage of a development continuum, to the best of our knowledge, this is the first time that both fertilization and pregnancy were achieved after halting the fertilization process long before the appearance of pronuclei (at ~4 hours after insemination).
Trial registration number: not applicable

P-186 Volumetric imaging provides insight into the 3D ultrastructural organization of maturing human oocytes

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Study question: Is volume electron microscopy suitable for high-resolution oocyte imaging in three dimensions (3D)?
Summary answer: Focused Ion Bean Scanning Electron Microscopy (FIB-SEM) allows 3D visualization and quantitative analysis of ultrastructural features in the large human oocyte volume.
What is known already: Transmission electron microscopy (TEM) has been traditionally used to study the fine morphology of female gametes. However, 2D micrographs provide only limited information about the topology of subcellular structures. Volumetric studies are needed to elucidate the 3D organization of complex oocyte cytoplasm.
Study design, size, duration: An academic study conducted in collaboration with an IVF clinic. Advanced 3D - ultrastructural analysis was performed on 9 human oocytes representing 3 stages of maturation (3 germinal vesicle (GV), 3 metaphase I (MI), 3 metaphase (MII) oocytes) collected from February 2018 to November 2019.
Participants/materials, setting, methods: Spare IVF oocytes, donated by 9 young egg donors (22-29 years), were cultured in vitro until they reached a defined developmental stage. Each oocyte´s meiotic status was determined based on the presence/absence of a prophase nucleus, a polar body, and a non-invasively detectable MI/MII spindle. Following standard TEM preparation, individual oocyte-containing resin blocks were coated with a thin carbon layer[JJ1] , mounted on the microscope stage, and subjected to FIB-SEM imaging.
Main results and the role of chance: FIB-SEM tomography provided an unprecedented view of the oocyte´s intracellular morphology. Automated serial scanning of newly exposed sample surface generated large stacks (120-1294 slices) of ultrastructural images with 40-100 nm z-resolution. The tomographic reconstruction of acquired datasets revealed the spatial arrangement of inner oocytes´ structures. The imaging protocol was optimized to ensure sufficient image detail, minimal noisiness, and time-efficiency of large volume scanning. Comparison of oocytes fixed at different maturation stages confirmed previous TEM observations that the cortical region of GV oocytes is deprived of membraneous structures, and major organelle redistribution occurs during the MI phase. Semi-automated 3D image segmentation was employed to distinguish distinct organelle populations and evaluated their abundance. Subsequent quantitative analysis of volumetric data showed that the mitochondrion occupies ~ 5.27 % of MII oocyte volume. In conclusion, the volumetric imaging, followed by advanced image analysis, maximizes the amount of morphological data obtained from a single human oocyte.
Limitations, reasons for caution: The imaging procedure was pioneered on a small number of hormonally-primed oocytes, which failed to complete development in vivo. There is a trade-off between resolution, the size of the 3D volume, and imaging time. Block-face ion milling during FIB-SEM imaging inevitably results in sample destruction.
Wider implications of the findings: This proof-of-concept study opens up new possibilities to study the delicate architecture of scarce human oocytes. Enhancing our knowledge of the spatial organization of ooplasm is pivotal for developing experimental and therapeutical strategies involving oocyte microsurgery.
Trial registration number: not applicable

P-187 Multinucleation and reverse cleavage, sings of early embryo self-repair mechanisms

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Study question: Have multinucleation and reverse cleavage any effect on embryo development and clinical outcomes on IVF treatments?
Summary answer: Embryos capable of repairing dysmorphisms and developing up to blastocyst stage keep intact their ability to become healthy babies.
What is known already: Time-lapse systems allow IVF laboratories to perform in-depth analysis of embryo development using the continuous monitoring tool. Some events that are impossible to detect with conventional morphologic evaluation, such as reverse cleavage or multinucleation, can be detected using time-lapse. Even though the low scientific evidence, the presence of these events is considered a negative factor when the embryo quality assessment is performed. However, it has been described the possibility that embryos have self-repair intrinsic methods.
Study design, size, duration: Retrospective study including data from 3,577 cycles with 21,274 embryos cultured until blastocyst stage using one-step culture media in time-lapse incubators (Embryoscope, Vitrolife) up to day 5/6 between 2014 and 2019.
Participants/materials, setting, methods: Three embryo groups were considered: Control group, embryos without multinucleation or reverse cleavage (CG; n=16,897); Multinucleation group, embryos with at least one blastomere multinucleated on D+2/3 (MNC; n=3,879) and Reverse Cleavage group, embryos undergoing complete fusion of two blastomeres on D+2/3 (RC; n=498). Single embryo transfer was performed on blastocyst stage. Clinical outcome rates were compared between groups and analyzed by Chi-square test.
Main results and the role of chance: As published by other groups, the 2.3% of our embryos showed at least one reverse cleavage event and we observed multinucleation in the 18.2% of the embryos. Blastocyst rate of dysmorphism groups was significantly lower ($p<0.05$) than Control group (MNC=20.0%; RC=27.7%; CG=58.0%). Once transferred, MNC and RC evolutive embryos showed significantly lower pregnancy (MNC=47.9%; RC=46.8%; CG=60.8%; $p<0.05$) and clinical pregnancy rates (MNC=39.4%; RC=40.4% CG=50.6%; $p<0.05$) than the Control group ($p<0.05$). However, during the post-implantational development the negative effect of dysmorphisms disappears, reaching values of live birth rate comparable to the Control group (MNC=28.3%; RC=31.9% CG=33.8%; p=0.17). These results prove the importance of blastocyst culture and the inherent capability of the embryos to overcome some abnormal dynamics as multinucleation and reverse cleavage. Thus, these embryos showing the poor-prognosis events can be considered for transfer or vitrify.
Limitations, reasons for caution: There is a wide difference on sample size between groups despite the fact that the statistical analysis considers that into account. There are some ongoing pregnancies in all groups.
Wider implications of the findings: When analyzing the development of embryos undergoing reverse cleavage and multinucleation, we hypothesize that these embryos could be showing a self-correction mechanism for some type of error detected. Embryos capable of repairing and developing up to blastocyst stage keep intact their ability to become healthy babies.
Trial registration number: not applicable

P-188 Ambient light intensity and wavelength in the IVF laboratory does not affect life birth rates

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Study question: Do different wavelengths and intensities in ambient lighting affect clinical outcomes?
Summary answer: Variations on ambient lighting intensity and wavelength do not affect life birth rates.
What is known already: Light is one of the factors to consider when designing an IVF laboratory. Most IVF clinics work under reduced illumination, trying to mimic uterine conditions as much as possible. Nevertheless, it has been described that 95% of the light that affects an embryo comes from the microscope, not ambient lighting.

It is well accepted that exposure to extreme lighting conditions affects embryos through photo-oxidation and the creation of reactive oxygen species. Still, there is no study that documents the effect of different wavelengths on human embryos.
Study design, size, duration: Prospective study performed between january 2019 and february 2020. Every 60 days we changed ambient illumination conditions using the LED lighting installed throughout the IVF laboratory. Six different groups were created: Cyan (470nm), Green (550nm), Yellow (600nm), Orange (625nm), intense white (WH), and low intensity white (WL) as control group.
Participants/materials, setting, methods: A total of 572 egg donation cicles with 355 fresh single embryo transfers were included in the study. In all cycles ICSI and Time-lapse culture was performed (*Embryoscope*, Vitrolife). PGT and testicular biopsy/aspiration treatments were excluded. Eggs and embryos were exposed to ambient illumination during pick-up, denudation, ICSI, and embryo transfer procedures.
Main results and the role of chance: Light exposure during embryo/gamete manipulation is inevitable. Hence, we analyzed parameters linked to the success

of an IVF cycle to assess the effect of different lighting conditions concluding that neither light color nor intensity affect IVF success rates.

No differences were found between groups regarding maternal age, age of the recipient, diagnostic, or number of eggs received (p>0.05). Fertilization rates were similar between groups (C= 77.04%; G= 73.72%; Y= 75.64%; O= 78.1%; WL=76.4%; WH=75.2%; p=0.216) as well as good quality blastocyst rates (C= 57.35%; G= 57.37%; Y= 62.30%; O= 59.75%; WL=63.28%; WH=60.55%; p=0.234). Regarding clinical outcomes both implantation and miscarriage rates were found to be equal between groups (C= 61.67%; G= 52.89%; Y= 55.10%; O= 66.18%; WL=66.00%; WH=53.55%; p=0.194, and C= 24.32%; G= 19.15%; Y= 11.11%; O= 24.44%; WL=15.15%; WH=8.11%; p=0.301). The main outcome for this study was live birth rates and no differences were found (C= 51.85%; G= 50.00%; Y= 52.17%; O= 53.97%; WL=57.14%; WH=50.75%; p=0.168).

Limitations, reasons for caution: We must take into account that embryos were cultured inside a time-lapse incubator, diminishing the effect of ambient light.

Wider implications of the findings: This study demonstrates that, with advances in culture technology, neither light intensity nor light wavelength affecting gametes/embryos during manipulation influence clinical outcomes.

Trial registration number: Not applicable

P-189 The transcriptional profile of arrested cleavage stage human embryos

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Study question: What are the molecular pathways overactivated in arrested cleavage stage human embryos?

Summary answer: There is an upregulation of mitochondrial activity and cellular stress-related pathways in arrested cleavage embryos, which is in agreement with the "quiet hypothesis".

What is known already: mtDNA content decreases during embryo development, however there is a high increase in mtDNA content in arrested cleavage embryos that may correspond to a response to intrinsic or extrinsic factor creating stress. This reasoning would be in agreement with stablished hypothesis showing that a basal metabolism with a moderate-low energy consumption is actually a sign of embryo health, so a viable embryo does not need to use an extra energy to accommodate to the environment (Leese, 2012). The study of the transcriptional profile during human embryo development will give further information about key molecular process involved in in-vitro embryonic competence.

Study design, size, duration: A prospective cohort study was performed with 11 MII oocytes (average age= 22,9 years), 10 non-arrested cleavage embryos (average age= 29,9 years), 5 arrested cleavage embryos (average age= 38,8 years) and 8 blastocysts (average age= 39,1 years). All specimens were warmed and sampled in PCR tubes with 2 µl of suitable reaction buffer for the RNA sequencing protocol.

Participants/materials, setting, methods: Specimens were analyzed by single-cell RNA sequencing (scRNA-seq). Correlation studies, principal component and differential expression analysis were performed with DESeq2 package. Differential gene expression analyses were done using the parametric Wald test, with Benjamini-Hochberg multiple test correction (padj). Finally, Fgsea algorithm was used for enrichment analysis on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene ontology (GO) terms.

Main results and the role of chance: We do not observe mitochondria-related activity pathways significantly (P>.05) deregulated between MII oocytes and non-arrested cleavage embryos considering GO and KEGG categories.

When comparing non-arrested cleavage embryos versus blastocysts, we observe several ATP production/consumption and cristae formation-related pathways significantly (P<.05) upregulated in blastocysts compared to non-arrested cleavage embryos considering GO and KEGG categories. This change in activity coincides with the metabolic activation event that occurs in the blastocyst stage.

However, when we analyze arrested cleavage embryos versus non-arrested cleavage embryos, we observe several ATP production related-pathways and mitochondria-related apoptosis pathways significantly (P<.05) upregulated in arrested cleavage embryos compared to non-arrested cleavage embryos considering GO categories. With KEGG categories, we notice a significant (P<.05) upregulation of oxidative phosphorylation in arrested cleavage embryos. On the other hand, when we analyze the differences between arrested cleavage embryos and blastocysts, taking into account the differences related to the change of embryo stage, we do not observe ATP production or consumption-related pathways significantly (P>.05) deregulated considering GO and KEGG categories. Then, human arrested embryos in parallel with the increase in mtDNA content, display an upregulation of mitochondrial activity and cellular stress which is in line with the expected overactive metabolism of non-viable embryos.

Limitations, reasons for caution: All analyzed blastocyst were aneuploid, so we are unable to determine what the results would be with euploid blastocysts. Also, although age can it be ruled out, no differences were observed between mean age from cleavage stage embryos (arrested and non-arrested ones) and blastocyst.

Wider implications of the findings: This study evidences the relation between extreme mtDNA content increase and the identification of the pathways involved in active metabolism and apoptosis in arrested cleavage stage human embryos.

Trial registration number: Not applicable

P-190 Can pregnancy be predicted according to mean infrared attenuated total reflectance (MIR-ATR) spectrometry of incubation medium of embryos in IVF?: A proof of concept

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Study question: Can MIR-ATR spectrometry of embryo incubation medium be used to predict embryo quality and IVF treatment results.

Summary answer: MIR-ATR spectrometry is able to distinguish between good and poor embryo quality and may improve the prediction of pregnancy based on better embryo selection.

What is known already: Infra Red (IR) spectra enable to determine if certain chemical functional components are present in a molecule. Attenuated total reflection (ATR) spectroscopy is a well established, simple and rapid technique for objective classification of biological samples. ATR spectrometry was investigated and confirmed to serve as an additional diagnostic tool in oral and gynecologic cancer. Currently, embryo quality is assessed based on morphokinetics parameters from Time-laps incubator and/or final embryo's grading

Study design, size, duration: Culture media of 227 embryos on cleavage stage and blastocyst stage were collected and analysed between January 1st, 2018 and December 31st 2020.

Participants/materials, setting, methods: The incubation medium liquid of embryos cultured in Time-lapse incubator were collected after transferring the embryos to their final destination. Infra-Red (IR) absorbance spectra was measured. Nominal resolution was 4 cm-1, wavenumber range was from 600 cm-1 to 5000 cm-1 and each absorbance spectrum was normalized to the height of amide I band at ~1650cm-1. Machine learning techniques utilized to build discrimination models for the absorbance data. Results were correlated with clinical and pregnancy results.

Main results and the role of chance: This preliminary study demonstrates that ATR spectrometry differs in media of : Day 3 embryos compared to day 5 embryos, top quality compared to poor quality embryos and implanting embryos comparing to non-implanting embryos. We found that MIR-ATR spectrometry might predict pregnancy in accuracy rates of 85%. MIR-ATR spectrometry of incubation medium can be used as an additional tool for selection of embryos for transfer.

Limitations, reasons for caution: Additional study and collection of larger number of culture Media is requested to validate MIR-ATR spectrometry as an additional tool in clinical set up in IVF units and laboratories.

Wider implications of the findings: MIR-ATR spectrometry can be used in the future as an additional tool for selection of embryos and prediction of cycles outcomes.

Trial registration number: NCT03317418

P-191 Time-lapse videography reveals morphometric and morphokinetic differences in the pronuclei of male and female human zygotes

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Study question: Do morphometric and morphokinetic profiles of pronuclei (PN) following intracytoplasmic sperm injection (ICSI) vary between male and female human zygotes?
Summary answer: Male and female zygotes displayed different PN morphometrics and morphokinetics. Additionally, variations were identified between sperm-originated (SPN) and oocyte-originated (OPN) pronuclei.
What is known already: Previous studies have investigated the use of PN-associated parameters via static observations as indicators of zygote viability, including size equality or juxtaposition. However, recent clinical application of time-lapse videography (TLV) provides a novel opportunity to assess these pronuclear events with greater accuracy and precision of morphometric and morphokinetic measurement. A number of recent TLV studies have also investigated potential live birth prediction by such PN associated measures, however whether or not there are gender associated differences in such measures which could in turn confound live birth prediction is unknown. Study design, size, duration: This retrospective cohort study included 94 consecutive autologous single day 5 transfer cycles (either fresh or frozen) performed between January 2019 and March 2020. Only ICSI cycles (maternal age <40 years) leading to a singleton live birth (43 males and 51 females) were included for analysis. All oocytes were placed in the EmbryoScope incubator for culture immediately post sperm injection with all annotation performed retrospectively by one embryologist (L-SO).
Participants/materials, setting, methods: Timings included 2nd polar body extrusion (tPb2), SPN(tSPNa)/OPN(tOPNa) appearance (differentiated by proximity to Pb2) and PN fading (tPNF). Morphometrics were evaluated at 8 (stage 1), 4 (stage 2) and 0 hour before PNF (stage 3), measuring PN area (um2), PN juxtaposition, and nucleolus precursor body (NPB) arrangement. Means ± standard deviation were compared using student t test or logistic regression as odds ratio (OR) and 95% confidence interval (CI), and proportional data by chi-squared analysis.
Main results and the role of chance: Logistic regression indicated that male zygotes had longer time intervals of tPb2_tSPNa than female zygotes (4.8±1.5 vs 4.2±1.0 h, OR=1.442, 95% CI 1.009-2.061, p=0.044), but not tPb2_tOPNa (4.7±1.8 vs 4.5±1.3 h, OR=1.224, 95% CI 0.868-1.728, p=0.250) and tPb2_tPNF (19.9±2.8 vs 19.1±2.3 h, OR=1.136, 95% CI 0.957-1.347, p=0.144). SPN increased in size from stage 1 through 2 to 3 (435.3±70.2, 506.7±77.3, and 556.3±86.4 um2, p=0.000) and OPN did similarly (399.0±59.4, 464.3±65.2, and 513.8±63.5 um2, p=0.000), with SPN being significantly larger than OPN at each stage (p<0.05 respectively). However, relative size difference between SPN and OPN was similar between male and female zygotes at 3 stages (33.6±61.7 vs 38.6±50.8 um2, p=0.664; 38.5±53.1 vs 45.7±71.9 um2, p=0.585; 38.4±77.4 vs 45.8±63.9 um2, p=0.615; respectively). More male than female zygotes reached central PN juxtaposition at stage 1 (77% vs 51%, p=0.010), stage 2 (98% vs 86%, p=0.048) and stage 3 (98% vs 86%, p=0.048). Furthermore, more OPN showed aligned NPBs than in SPN at stage 1 (45% vs 29%, p=0.023), but similar proportions at stage 2 (64% vs 50%, p=0.056) and stage 3 (76% vs 72%, p=0.618). There were no gender associated differences detected in NPB alignment in either SPN or OPN (p>0.05 respectively).
Limitations, reasons for caution: The retrospective design does not allow for control of unknown confounders. Sample size is considered relatively small. PN area measurement may not truly represent volume as PN may not be perfectly spherical. Findings were based on women <40 years old so may not apply to older population.
Wider implications of the findings: These findings augment and extend previous studies investigating PN parameters via static observations. The reported variations between male and female embryos may confound live birth prediction when using pronuclei morphometrics and morphokinetics. Larger scaled studies are warranted to verify these findings.
Trial registration number: Not applicable

P-192 Efficacy of postponement of intracytoplasmic sperm injection timing after spindle visualization for Metaphase I oocytes

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Study question: Does postponement of intracytoplasmic sperm injection (ICSI) timing after spindle visualization for Metaphase I (MI) oocytes improve developmental outcomes of embryos?
Summary answer: Postponement of ICSI timing after spindle visualization for MI oocytes improves blastocyst utility rates.
What is known already: Immature oocytes are generally considered poor developmental outcomes. Meanwhile, the timing of ICSI adjusted by using spindle visualization can improve clinically utilized embryos and live birth rates, but these outcomes remain inferior to those of mature oocytes. In in vitro maturation culture, nuclear maturation is thought to occur before the completion of cytoplasmic maturation, and in immature oocytes, synchronization of nuclear and cytoplasmic maturation may be insufficient for ICSI immediately after spindle visualization.
Study design, size, duration: Data for this retrospective cohort study were obtained 672 oocytes retrieved under mild stimulation cycles using letrozole, in patients aged younger than 39 years between April 2017 and October 2020. Written informed consent was obtained from all patients. This study was approved by the institutional review board.
Participants/materials, setting, methods: As a control group, 464 Metaphase Ⅱ oocytes that underwent ICSI immediately after visualization of the spindle were used. In group A, 103 MI oocytes underwent ICSI immediately after the first polar body release and spindle visualization, and in group B, 105 oocytes underwent ICSI 2-3 hours after spindle visualization. The primary outcomes were fertilization rates, degeneration, cleavage, embryo blastocyst formation, and utility rates. Outcomes were compared among the three groups.
Main results and the role of chance: The baseline fertilization rates of each group (control, A, B) were 82.3% (382/464), 73.8% (76/103), and 83.8% (88/105), respectively. The rate was significantly lower in group A than in the control group (P<0.05), and also tended to be lower in group A than in group B, although the difference was not significant. There was no significant difference in abnormal fertilization rates, oocyte degeneration rates, cleavage rates, and blastocyst formation rates among the three groups. [control, A, B: abnormal fertilization rate: 4.3% (20/464), 8.7% (9/103), 4.8% (5/105); oocyte degeneration rates: 3.0% (14/464), 1.9% (2/103), 3.8% (4/105); cleavage rates: 95.6% (307/321), 93.8% (61/65), 98.7% (74/75); blastocyst formation rates: 58.6% (177/302), 51.7% (31/60), 55.4% (41/74), respectively]. The blastocyst utility rates of control group and group B were significantly higher than in group A [41.7% (126/302), 45.9% (34/74), 26.7% (16/60), respectively] (P<0.05). There were no significantly different outcomes between the control group and group B.
Limitations, reasons for caution: The optimal timing of ICSI for MI oocyte cannot be determined by the presence or absence of spindles. In addition, the postponement duration we set was based on reports which reported on final oocyte maturation, and further investigation is needed to establish the optimal ICSI timing for MI oocytes.
Wider implications of the findings: In MI oocytes, postponement of ICSI timing after spindle visualization is essential for synchronization of the nucleus and cytoplasmic maturation.
Trial registration number: none

P-193 First cleavage division perpendicular to the pronuclear axis adversely affects the clinical outcome in human embryos

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Study question: Does the direction of formation for the first cleavage plane relative to the pronuclear axis affect clinical outcome?
Summary answer: A first cleavage division perpendicular to the pronuclear axis adversely affects the rate of embryo utilization for transfer or cryopreservation and the pregnancy outcome.
What is known already: It remains unclear how the first cleavage plane is determined in human embryos. By using time-lapse monitoring, our previous study (presented in ESHRE 2019) suggested that both the axis and locations of male and female pronuclei are involved in determining the first embryonic cleavage plane. Furthermore, by using immunofluorescence analysis, it was also revealed that most analyzed zygotes showed two pericentrin signals aligned around the interface between the male and female pronuclei. Our findings suggest that the pronuclear axis strongly influences the positions of the centrosomes, which become mitotic spindle poles and define the first cleavage plane. Study design, size, duration: From January 2015 to December 2017, time-lapse imaging (EmbryoScope®) of 3397 intracytoplasmic sperm injection (ICSI) oocytes was conducted. Of those, the relationship between the pronuclear axis and the first cleavage plane was analyzed in 607 normally fertilized embryos that cleaved to two cells and were obtained in 2015. Furthermore, of 3397 ICSI oocytes, 749 transferred embryos were classified based on the first cleavage patterns relative to the pronuclear axis, and the pregnancy rate was examined.
Participants/materials, setting, methods: A straight line connecting the centers of the pronuclei was defined as the 2PN axis. Based on the direction of the first cleavage relative to the 2PN axis, embryos were classified into three groups: parallel, perpendicular and intermediate. Fresh embryos were transferred on Day 2/3 (fresh-ET). Frozen and thawed embryos were transferred on Day 2/3 or Day 5 (F/T-ET). Clinical pregnancy was defined as confirmed gestational sac in the uterine cavity.
Main results and the role of chance: Of 607 analyzed embryos, 506 produced suitable images and were assigned to one of three groups: parallel (84.4%, n=427), perpendicular (9.7%, n=49) and intermediate (5.9%, n=30). Embryos that formed a cleavage furrow parallel to the 2PN axis were significantly more frequent than others (perpendicular, intermediate) (P <0.001). The embryo utilization rate for transfer or cryopreservation was significantly lower in the perpendicular group than in the parallel group (30.7% vs. 69.3%, P <0.01). Furthermore, of 749 transferred embryos, 504 assigned to the parallel and perpendicular groups were selected (n=470 and n=34, respectively), and the pregnancy outcome was analyzed. The mean maternal age was not significantly different between groups. The pregnancy rate of embryos was 24.2% (n=45/186) from fresh-ET and 39.4% (n=112/284) from F/T-ET in the parallel group, and 0% (n=0/14) from fresh-ET and 15.0% (n=3/20) from F/T-ET in the perpendicular group. Regardless of the types of embryo transfer (fresh or F/T), the pregnancy rate was significantly lower in the perpendicular group than in the parallel group (P <0.01). In addition, one of three patients who became pregnant from the transfer of an embryo in the perpendicular group had a miscarriage.
Limitations, reasons for caution: Since only ICSI embryos were analyzed in this study, the influence of fertilization methods on subsequent development could not be investigated. Further studies including preimplantation genetic testing for aneuploidy may help determine the reasons why pregnancy rates differ between groups.
Wider implications of the findings: We suggest that the 2PN axis is essential for determining the first cleavage plane because it seems to be involved in positioning the mitotic spindle poles. The direction of the first cleavage plane relative to the 2PN axis can be an important indicator for predicting embryo development and pregnancy outcome
Trial registration number: none

P-194 Impact of cryopreservation duration on pregnancy outcomes of vitrified-warmed blastocysts transfer using an open-device system

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Study question: Is there a negative effect of long-term cryopreservation upon pregnancy outcomes after transfer of vitrified-warmed blastocysts stored in an open-device system?
Summary answer: Prolonged cryopreservation of vitrified blastocysts up to 24 months increased the incidences of clinical pregnancy, ongoing pregnancy, and live birth, while decreased early miscarriage rate.
What is known already: Vitrification is adopted as the dominant approach for cryopreservation of human oocytes and embryos. However, little is known about the potential effect of prolonged storage after vitrification on the genomic integrity and metabolism of embryos. Several studies have sought to decipher the effect of cryopreservation duration on IVF pregnancy outcomes, but few were confined to vitrification and the results were inconsistent.
Study design, size, duration: This retrospective study included 6722 patients undergoing their first vitrified-warmed blastocyst transfer (VBT) cycles from January 2015 to June 2019 in a single fertility center in South China. The study was approved by the hospital's Ethics Committee.
Participants/materials, setting, methods: A total of 6722 eligible patients were divided into five groups according to the storage duration after vitrification: Group I: 0-3 months; Group II: 3-6 months; Group III: 6-12 months; Group IV: 12-24 months; Group V: 24-36 months. The IVF pregnancy outcomes were compared between groups. Multivariate logistic regression was conducted to evaluate the independent effect of storage duration on pregnancy outcomes.
Main results and the role of chance: The odds of clinical pregnancy outcomes were similar from Group I to 4. However, the chance of clinical pregnancy (Group I as reference; Group 2: adjusted odds ratio (aOR)= 1.04, 95% CI 0.93-1.17; Group 3: aOR = 1.02, 95% CI 0.84-1.25; Group 4: aOR = 0.93, 95% CI 0.66-1.31; Group 5: aOR = 0.54, 95% CI 0.38-0.76) , ongoing pregnancy (Group 2: aOR=0.99, 95% CI 0.89-1.11; Group 3: aOR = 0.94, 95% CI 0.77-1.14; Group 4: aOR = 0.87, 95% CI 0.62-1.22; Group 5: aOR = 0.41, 95% CI 0.29-0.60), and live birth rate (Group 2: aOR=1.00, 95% CI 0.89-1.12; Group 3: aOR = 0.98, 95% CI 0.81-1.19; Group 4: aOR = 0.91, 95% CI 0.65-1.27; Group 5: aOR = 0.46, 95% CI 0.32-0.66) significantly decreased, while the early miscarriage rate (Group 2: aOR=1.11, 95% CI 0.92-1.35; Group 3: aOR =1.25, 95% CI 0.92-1.70; Group 4: aOR = 1.33, 95% CI 0.77-2.31; Group 5: aOR = 2.42, 95% CI 1.36-4.31) significantly increased as the storage duration increased up to 24-36 months.
Limitations, reasons for caution: The primary limitation of this study was its retrospective nature. Besides, as all these data come from a single IVF treatment center, the results should be confirmed by a larger multicenter study.
Wider implications of the findings: Our study provides more evidence about the negative impact of long-term storage of vitrified embryos on the clinical outcome. Clinicians should adapt FET strategies based on the embryo storage duration.
Trial registration number: not applicable

P-195 The influence of hormones and initial cell number on the size of self-assembled embryo-like structures

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Study question: Do hormonal treatments and initial cell number influence the formation of embryo-like structures (ELS) during their development in regard to size?
Summary answer: The chosen initial cell number for ELS-assembly seems to influence the ELS size only until day 4, while hormones affect embryo size throughout their development.
What is known already: The initial cell number is an important parameter for the development of ELS, which might help to better understand how embryos regulate their size. Previous studies on differently sized natural murine embryos revealed that an initial difference in size at the early stage is compensated until E6.75. Normal-size embryos experience an increased mitotic activity before E6.75, whereas larger sized embryos show an increased apoptotic activity, indicating an important control point of cell turnover by adapting mitotic activity and cell survival. Embryo development is strongly dependent on appropriate β-estradiol and progesterone levels.
Study design, size, duration: The first set of experiments interrogated the influence of initial cell number (two conditions) on the size of formed ELS during the first 3 days (D1-3). The second set included two different hormonal treatments and the two conditions of initial cell number (the same as in the first experiments) generating four different groups. For each day one Aggrewell

(generating 1200 ELS/well) per condition was harvested. Experiments were repeated at least three times.
Participants/materials, setting, methods: ELS are generated by self-assembly in microwell-chamber plates combining embryonic stem cells, trophoblast stem cells and extraembryonic endoderm stem cells. Cells were cultured with and without addition of β-estradiol and progesterone, starting with different initial cell numbers (106 vs. 42 cells/ELS). ELS were harvested, stained, and at least 40 randomly picked ELS per condition were measured and statistically analyzed with Two-way ANOVA and Tukey's multiple comparison test. Results show the average area ± SD.
Main results and the role of chance: The results show a continuous increase in the size of ELS during the first three days of cultivation, with significant lower values (on D1-D3) when ELS were assembled from 42 initial cells (D1: 224.1±87.7 µm²; D3: 674.0± 84.4 µm²) compared to ELS formed with 106 initial cells (D1: 467.1±224.1 µm²; D3: 1275.0±348.0 µm²). Onward on the course of self-assembly, ELS with 42 initial cells were still smaller on D4 (1465.7±657.6 µm²) compared to ELS formed with 106 initial cells (2028.6±522.4 µm²). However, these differences could not be measured on D5 (106 initial cells: 1892.2±603.7 µm²; 42 initial cells: 1855±448.5 µm²), D6 (106 initial cells: 2143.3±622.1 µm²; 42 initial cells: 1788.4±585.5 µm²) and D7 (106 initial cells: 2146.7±628.1 µm²; 42 initial cells: 2319.5±778.8 µm²). Differences between the conditions with and without hormonal treatments (HT) could also be detected especially when ELS were generated with 42 cells: on D4 ELS with HT (1730.4±852.4 µm²) were significantly larger than without hormones (1201.2±462.9 µm²). In contrast, on D7 HT influenced the size of ELS distinctly depending on the initial cell number (42 cells: 1989.2±558.3 µm² with HT vs. 2649.7±999.4 µm² without HT; 106 cells: 2334.9±770.2µm² with HT vs. 1958.6±486.1 µm² without HT).
Limitations, reasons for caution: An even cell distribution is crucial for reproducible ELS-formation. Unfortunately, the used techniques for cell seeding led to an uneven distribution within the microwells. Moreover, different orientation of ELS during the size assessment might be an additional reason for the high variance of ELS size within one condition.
Wider implications of the findings: Even if the results seem to be in accordance with the observations made with natural embryos regarding compensation of size until E6.75, additional experiments need to be conducted. Further investigations should be carried out by testing different culture formats to obtain a more even cell distribution during the cultivation.
Trial registration number: Not Applicable

P-196 Bacterial influence on oocyte quality - the secret of a successful fertilization

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Study question: Is there a difference in bacterial composition of follicular fluid between oocytes developing a good quality blastocyst and oocytes that fail fertilization?
Summary answer: Follicular fluids of oocytes failing fertilization show a different bacterial profile compared to follicular fluids of oocytes that were successfully fertilized.
What is known already: The presence of pathogens in the female reproductive tract has been intensively investigated. *Lactobacillus* species are mainly associated with a healthy genital tract and good prognosis for a successful pregnancy. Studies of the bacterial composition of follicular fluids have been mainly undertaken in women participating in reproductive medicine treatment because of the nature to obtain the specimen. In most studies follicular fluids have been pooled for analysis. Information on separately collected follicular fluids is still rare. We hypothesized that the composition of bacteria within follicular fluids is responsible for the success of the fertilization process.
Study design, size, duration: The study was designed and conducted at the Kinderwunsch Institut Schenk GmbH (Dobl, Austria) together with DNA-Technology. Follicular fluids from 46 patients undergoing IVF (*in vitro* fertilization) and ICSI (intracytoplasmic sperm injection) treatment were included and analyzed.
Participants/materials, setting, methods: Follicular fluids from 46 patients were collected separately. 2 follicular fluids from each patient were screened for common bacteria of the genital tract. One from an oocyte developing a good quality blastocyst and one displaying fertilization failures. Samples were analyzed for bacterial composition using the Femoflor16 (DNA-Technology).
Main results and the role of chance: Quantitative analysis revealed a higher total bacteria mass in follicles from oocytes that failed fertilization. Furthermore, *Lactobacillus* were not present in those follicles compared to good blastocyst follicles. In addition, *Chlamydia trachomatis* was found mainly in follicular fluid of not fertilized oocytes together with *Eubacterium*, *Gardnarella* and *Trichomonas species*. Interestingly, a trend of elevated levels of *Ureaplasma* species in follicular fluids of oocytes developing good quality blastocysts was observed.
Limitations, reasons for caution: Contamination of follicular fluids due to the procedure of oocyte pick up and follicular fluid retrieval cannot be completely excluded. Results should be confirmed with a higher sample size.
Wider implications of the findings: We assume that different bacterial compositions in follicular fluids are responsible for the destiny of the oocyte. It is tempting to speculate that bacterial analysis of follicular fluids may be beneficial to select to best oocytes in future IVF/ICSI treatments.
Trial registration number: not applicable

P-197 Two different strategies for embryo culture and selection: time-lapse with single-step medium and conventional incubator with sequential media. Are there differences in clinical results?

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Study question: Is there a significant difference in the clinical results of embryos cultured in time-lapse systems with single-step medium and conventional benchtop incubators with sequential media?
Summary answer: Embryos cultured in time-lapse systems and single-step media are more likely to achieve an ongoing pregnancy and have higher implantation rates than those cultured otherwise.
What is known already: One of the strategies for embryo culture in IVF consisted in conventional benchtop incubators combined with sequential culture media (CI-Seq). New generation time-lapse systems provide useful information on the morphokinetics of embryo development, but also a stable culture environment where embryos can develop undisturbed until blastocyst stage when paired with single-step culture media (TLS-SS). These features have the potential to improve embryo development and selection. Nonetheless, there is inconclusive evidence of whether this new culture strategy has a significant effect on clinical results of ICSI treatments. Studies on the matter are heterogeneous and reduced in both number and sample size.
Study design, size, duration: Unicentric retrospective cohort study. We compared the results of 11471 blastocyst transferences from 10276 ICSI treatments performed during 4 consecutive years, where embryos were cultured either on CI with sequential media (N=5255) or a TLS with single-step medium (N=5021). 3922 of the totals were fresh embryo transfers (ET) and 7549 frozen-thawed ET. We compared the implantation rate (IR) and ongoing pregnancy rate (OGPR) in both study groups, stratifying by ovum origin.
Participants/materials, setting, methods: Three models of TLS were used for embryo culture: EmbryoScope, EmbryoScope Plus (Vitrolife) and GERI (Genea Biomedx), as well as one CI (ASTEC). Sequential media: Cook, Origio, Vitrolife; Single-step media: Gems, Irvine, Life Global. Embryo scoring and selection was performed by ASEBIR criteria in the CI group, and by morphological and morphokinetic assessment for embryos cultured in TLS. Embryos were extracted from the CI only for media change. Statistical analysis: ANOVA tests and Logistic regressions.
Main results and the role of chance: A general Logistic Regression was performed, including egg origin, PGT-A and culture strategy to explain their impact in OGPR. Egg origin (OR=1,094 (95%CI: 1,015-1,179); *P*=0,019) and culture strategy (OR=1,141 (95%CI: 1,060-1,229); *P<0,001*) were statistically significant, which confirms the need for stratification due to the heterogeneity of the groups. The total IR in the TLS-SS group was 54,68±48,84%, significantly higher than that of CI-Seq (49,18±47,91 %; *P*<0,001). In ovum-donation treatments, a complete Logistic Regression for OGPR, with all typical confounding variables (age, BMI, n° oocytes, fresh/frozen transfer, number and day of ET)

resulted in an OR=1,187 (95%CI: 1,074-1,313; *P*=0,001) favoring culture in TL-SS. IR in these treatments were 61,98±47,68% in TL-SS vs 55,08±46,58% in CI-Seq (*P*<0,001) in fresh transfers and 51,48±48,91% in TL-SS vs 44,39±47,67% in CI-Seq (*P*<0,001) in frozen-thawed ET. In autologous treatments with PGT a similar regression yielded an OR=1,055 (95%CI: 0,889-1,252; *P*=0,542) for culture strategy. The IR of genetically tested ET was not significantly different: 53,08±49,49% for TL-SS, 50,90±49,07% for CI-Seq, *P*=0,246. In autologous procedures without PGT, culture strategy was not significant for OGPR (OR=0,998 (95%CI: 0,835-1,191), *P*=0,979) nor IR of fresh (49,75±48,91% TL-SS vs 44,23±47,36% CI-Seq; *P*=0,081) nor frozen-thawed transferences (50,77±48,33% TL-SS vs 50,67±47,33% CI-Seq; *P*=0,970).

Limitations, reasons for caution: After fertilization check, embryos were evaluated exclusively on D5/6. On D3, embryos cultured in CI were taken out only for a quick media change, but not for evaluation, and all handling was done in isolette cabins with controlled environmental conditions. Being a retrospective study, there is high variability in population.

Wider implications of the findings: A more homogenous prospective study, including comparison in life-birth rates, is necessary to extract final conclusions. However, our results suggest that the introduction of TLS and SS media in IVF laboratories might be a valid strategy to increase clinical results, especially in fresh embryo, thanks to an improved embryo selection.

Trial registration number: not applicable

P-198 Higher rate of direct uneven cleavage (DUC) embryos in women exhibiting high ovarian response

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Study question: Does high ovarian response to controlled ovarian stimulation (COS) have a negative effect on oocyte quality?

Summary answer: High ovarian response is associated with reduced oocyte quality manifested as higher fraction of immature oocytes and higher rate of direct uneven cleavage (DUC) embryos.

What is known already: The literature regarding the effect of ovarian hyperstimulation on oocyte quality is limited and controversial. Results from several studies suggest that hyper response to controlled ovarian stimulation has a detrimental effect on oocyte and embryo quality, while others failed to confirm the existence of a direct toxic effect on oocyte and embryo quality. The association between temporal embryonal milestones and implantation rate has been previously demonstrated ,offering an additive tool by which oocyte quality, represented by embryo dynamics, can be evaluated. None of the aforementioned studies, however, used time lapse monitoring (TLM) system to evaluate oocyte and embryo quality.

Study design, size, duration: This study included a retrospective assessment of morphokinetic parameters performed by TLM from three university affiliated medical centers between January 2014 and December 2019. The developmental process and kinetics of 1863 embryos obtained from the study group, referred as the " high ovarian response" (HOR) group, was compared to 4907 embryos from the control group - the "normal ovarian response" (NOR) group.

Participants/materials, setting, methods: The study included patients younger than 38 years who underwent COS with consecutive aspiration of either more than 15 oocytes (the HOR group) or 6-15 oocytes (the NOR group). A comparison was made between the groups regarding morphokinetic parameters, including the rate of embryos manifesting direct uneven cleavage (DUC) at first cleavage (DUC-1), as well as implantation and pregnancy rates. Logistic regression was conducted to assess the association between patients' characteristics and implantation rate.

Main results and the role of chance: Oocyte maturation rate was significantly lower, and the DUC-1 embryos rate was significantly higher in the high ovarian response group compared to the normal ovarian response group (56.5% Vs 90.0%, p<0.001 and 16.2% Vs 12.0%, p=<0.001; respectively). Following the exclusion of DUC-1 embryos, embryos from the HOR and the NOR groups reached the consecutive morphokinetic milestones at a similar rate and demonstrated similar implantation and clinical pregnancy rates. In a multivariate analysis preformed, only maternal age was found to be predictive for implantation.

Limitations, reasons for caution: The groups were not homogenous in their basic characteristics. Important information regarding the maximal dose of GT obtained, previous IVF response and ovarian reserve testing was lacking

Wider implications of the findings: Higher oocyte quantity might have an effect on oocyte quality manifested as higher fraction of incompetent oocytes and higher rate of DUC-1 embryos. Once beyond the preliminary developmental stages, embryos from both groups reach the morphokinetic milestones at a similar rate and display similar implantation and pregnancy rates.

Trial registration number: not applicable

P-199 A case report to suggest that there must be other mutations than PATL2 or TUBB8 to cause oocyte maturation arrest

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Study question: What causes our patient's repeated almost complete oocyte maturation arrest (OMA)?

Summary answer: Since we did not detect PATL2 and TUBB8 mutations, both known to cause OMA, this case was likely caused by mutations in HUS1 and ITGB3

What is known already: OMA has been associated with loss-of-function in key genes, such as PATL2 and TUBB8. Such patients have, however, uniformly have been unable to conceive with IVF

Study design, size, duration: We here report the case of repeatedly presenting patient between 2009 until 2020 (age 30 at 1st and 41 at last visit).

Participants/materials, setting, methods: The couple underwent 7 IVF treatments under several ovarian stimulation protocols at different gonadotropin dosages and in different preparations to try to recruit mature eggs. She conceived in her 2nd IVF cycle in 2009 and delivered uneventfully in 2010. She then conceived spontaneously and delivered a healthy boy in 2014. The couple since then has been attempting another pregnancy. Remarkably, in all IVF cycles all eggs but one arrested at prophase.

Main results and the role of chance: The female demonstrates abnormally high ovarian reserve for age (AMH=5.9 ng/mL in 2019) (mean, 10.6 oocytes). In all cycles, all but one retrieved were immature. In vitro maturation rate for the GV oocytes was 28%. Resultant M2s, however, demonstrated morphological abnormalities, such as giant polar bodies. *In vivo* M2s, in contrast, were always morphologically unremarkable, and their fertilization rate was 85%. Embryo morphology deteriorated appreciatively with advancing age. Sanger sequencing for TUBB8 and PATL2 genes were unremarkable. Whole genome sequencing of her and her sister (who had no fertility problems) revealed mutations of genes belonging to the integrin family (ITGB3) and DNA repair checkpoint (HUS1), both of which could be determinants in the observed maturation arrest.

Limitations, reasons for caution: A functional study, coupled with imaging of the discarded material, will likely offer further information regarding the mechanisms leading to OMA in this female.

Wider implications of the findings: This case report represents a new phenotype of female infertility, characterized by almost complete maturation arrest which, however, still offers opportunity for pregnancy. Further isolation of underlying mutation(s) may offer additional insights about checkpoints required for the transition of prophase to metaphase in human oocytes.

Trial registration number: NA

P-200 To transfer or to discard: A retrospective analysis of ploidy, implantation and birthweight outcomes of grade "C" blastocysts following preimplantation genetic testing for aneuploidy (PGT-A)

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Study question: What's the ploidy status of grade "C" blastocysts and what are their implantation potential and birthweight outcomes when tested euploid?
Summary answer: Grade "C" blastocysts were less likely to be euploid compared to grades "A/B". Euploid "C"s led to reduced but reasonable implantation potential with similar birthweights.
What is known already: In contrast to grade "A" or "B", grade "C" blastocysts are generally considered borderline quality in most *in vitro* fertilization programs, with inconsistent policies between clinics. Little evidence has been reported regarding their euploidy rate, implantation potential, and birthweight outcomes.
Study design, size, duration: This retrospective cohort study included 426 consecutive autologous-oocyte patients undergoing PGT-A (biopsy at day 5/6) at two associated private clinics between January 2013 and August 2020. A total of 1418 resulting blastocysts (tested either euploid or aneuploid) were analysed. Implantation outcomes were assessed in a subset of 520 singly transferred euploid blastocysts. Birthweight outcomes were evaluated in 209 singleton newborns using a gestation-adjusted Z score taking into account gestational age and baby gender.
Participants/materials, setting, methods: Blastocysts were graded "A/B/C" according to a combination of inner cell mass and trophectoderm morphology. Endpoints included ploidy, implantation and birthweight outcomes. Multiple regression (logistic or linear) was performed to investigate relative prognosis of grade "C" blastocysts using different endpoints in reference to grade "A/B" blastocysts, expressed as either adjusted odds ratio (aOR) or coefficients (β) with 95% confidence intervals (CI). Maternal age and biopsy day (5/6) were included as potential confounders at regression analysis.
Main results and the role of chance: Grade "C" blastocysts (n=466) were associated with a lower euploidy rate in reference to either grade "A" (n=179, aOR=0.412, 95% CI: 0.278-0.611, P=0.000) or "B" blastocysts (n=773, aOR=0.535, 95% CI: 0.418-0.685, P=0.000). Euploid "C" grade blastocysts (n=128) led to significantly reduced chance to implant when compared to either grade "A" (n=90, aOR=0.387, 95% CI: 0.215-0.696, P=0.002) or "B" blastocysts (n=302, aOR=0.617, 95% CI: 0.404-0.944, P=0.026); although implantation rate was still considered at a reasonable level (44.5%) as opposed to grades "A" (66.7%) or "B" (57.6%). However, no significant difference was observed in the birthweight (g, mean ± standard deviation) following the transfer of a single euploid grade "C" blastocyst (n=42, 3310.8±704.1) in comparison to a single euploid grade "A" (n=48, 3367.8±519.3, P>0.05) or "B" blastocyst (n=119, 3284.5±535.5, P>0.05). Taking into account maternal age, biopsy day, gestational age and baby gender; further multiple linear regression analysis also showed similar results using either birthweight itself (C vs A, β=-52.395, 95% CI: -148.83–43.893, P=0.282; C vs B, β=-104.338, 95% CI: -272.653–63.977, P=0.223), or the gestation-adjusted Z score as an endpoint (C vs A, β=0.101, 95% CI: -0.001–0.164, P=0.052; C vs B, β=0.084, 95% CI: --0.073 – 0.241, P=0.290).
Limitations, reasons for caution: The retrospective design of this study does not allow control for unknown confounders. Inner cell mass or trophectoderm was not graded separately making it difficult to further break down the "C" grade blastocysts. Only blastocysts suitable for biopsy were included for analysis, so results may not extrapolate to untested blastocysts.
Wider implications of the findings: Grade "C" blastocysts may still hold its clinical value despite reduced euploidy rate. PGT-A may be considered as a potential approach to utilize grade "C" blastocysts more effectively, without affecting birthweight outcomes. This is also potentially useful in patient counselling.
Trial registration number: not applicable

P-201 The beneficial effects of ZP-free culture on cytoplasmic fragmentation in human embryos. : An innovative trial using 3PN zygotes

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Study question: Is it possible to culture ZP-free embryos to eliminate perivitelline threads, which are known to be involved in generating cytoplasmic fragments at the first cleavage?
Summary answer: ZP-free culturing, an innovative system that decreases the amount of cytoplasmic fragments without disrupting the blastomeres, using incubators with time-lapse imaging.
What is known already: A study in 2017 observed perivitelline threads in more than 50% of cleavage-stage human embryos using time-lapse imaging, and the rate of cytoplasmic fragmentation (at the first cleavage) was significantly decreased in embryos without perivitelline threads ($P < 0.001$). While it has been proposed that perivitelline threads play an important role in crosslinking the cumulus cells and oocyte during maturation, the mechanism underlying such a role remains unclear. It is also unknown whether the threads still function in mature MII oocytes.
Study design, size, duration: A prospective study was conducted using 2,852 normal (2PN/2PB) embryos from c-IVF/ICSI and 113 abnormal (3PN) embryos obtained from c-IVF between 2017 and 2019. The zona pellucida (ZP) of 71 abnormal embryos was removed at the pronuclear stage ("ZP-free"), and the rest (n=42) were cultured as "ZP-intact". Normal and abnormal embryos were cultured for five days in bench-top incubators (MINC, COOK) and an incubator equipped with a time-lapse imaging system.
Participants/materials, setting, methods: Embryos used in this study were donated by 412 couples who underwent c-IVF cycles in our clinic between 2017 and 2019. For ZP removal, 3PN embryos were placed in 0.125M sucrose-containing HEPES media drops to reduce the ooplasm size. Then, ooplasms were completely separated from ZPs by a laser and pipetting. Embryo development and morphology of the three groups (normal, ZP-intact and ZP-free abnormal) were compared based on the degree of cytoplasmic fragmentation.
Main results and the role of chance: The first cleavage occurred in 97.8% (n=2,790/2,852) of 2PN/2PB, 83.3% (n=35/42) of ZP-intact 3PN and 97.2% (n=69/71) of ZP-free 3PN. Normal (2PN/2PB), ZP-intact and ZP-free 3PN embryos were classified into three groups based on the modified Veeck's criteria thus: <20% fragmented compared to the total volume of cytoplasm at the first cleavage (Grade 1 and 2, Good); 20-39% fragmented (Grade 3, Fair) and ≧40% fragmented (Grade 4, Poor). Of 69 cleaved ZP-free 3PN embryos, 68.1% (n=47) showed less than 20% fragments which was significantly higher than 2PN/2PB (43.7%, n=1,218/2,790) and ZP-intact 3PN (45.7%, n=16/35; $P < 0.05$). Furthermore, 24.6% (n=17/69) of ZP-free 3PN embryos showed 20-39% fragments which was significantly lower than 2PN/2PB (45.9%, n=1,281/2,790; $P < 0.05$). In addition, 50.7% of ZP-free 3PN embryos (n=36) developed to the morula stage after the third cleavage, and 29.6 % (n=21) formed blastocoel and became blastocysts. Thus, removing the ZP before the first cleavage did not adversely affect embryo development and decreased the cytoplasmic fragmentation.
Limitations, reasons for caution: Due to ethical and clinical limitations, we only examined abnormally fertilized embryos in this study. Moreover, since the relationship between the perivitelline threads and cytoplasmic fragments is unclear, we plan to conduct molecular biological analysis of the perivitelline threads in further studies.
Wider implications of the findings: This study revealed that ZP is not always necessary after the pronuclear stage because ZP-free embryos studied herein developed normally and maintained cell adhesion well. This innovative culture method might provide the breakthrough needed for patients to improve embryo quality who obtain embryos with severe fragmentation caused by perivitelline threads.
Trial registration number: not applicable

P-202 Past embryo viability is not always a good predictor of future pregnancy: dynamic viability suggests video has limited benefit over static images for AI assessment

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Study question: Does embryo quality/viability change over time, suggesting the use of video for AI-based embryo quality assessment has limited benefit over single point-in-time images?

Summary answer: AI assessment of single static embryo images at multiple time-points indicates embryo viability is dynamic, and past viability is a limited predictor of future pregnancy.

What is known already: Artificial Intelligence (AI) has been applied to the problem of embryo quality (viability) assessment using either video or single static images. However, whether historical data within video provide an additional advantage over single static images of embryos (at the time of transfer) for assessing embryo viability is not known. This applies to both manual and AI-based embryo assessment. If embryo viability changes over time prior to transfer, then the implication is that the assessment of future pregnancy using historical embryo data from videos would provide limited additional value over single static images taken immediately prior to transfer.

Study design, size, duration: Retrospective dataset of single embryo images taken at up-to three time-points prior to transfer: Early Day 5, Late Day 5 (8 hours later), and Early Day 6 (16 hours later), with corresponding fetal heartbeat (pregnancy) outcomes. The AI assessed the viability of each embryo at its available timepoints. Viability prediction was compared with pregnancy outcome to assess viability predictiveness at each timepoint prior to transfer, and assess the variability of viability over time.

Participants/materials, setting, methods: Single static images of 173 embryos were taken using time-lapse incubators from a single IVF clinic. 116 embryos were viable (led to a pregnancy) and 57 were non-viable (did not lead to a pregnancy). The AI was trained on thousands of Day 5 static embryo images taken from multiple IVF laboratories and countries, but was not trained on data from this clinic.

Main results and the role of chance: When embryos were assessed as viable by the AI immediately prior to transfer (no delay), the AI accuracy (sensitivity) in predicting pregnancy was 88.1% (59/67) for Early Day 5, 84.8% (28/33) for Late Day 5 and 87.5% (14/16) for Early Day 6. When the delay between AI assessment and transfer is 8 hours, 16 hours and 24 hours, the the accuracy drops to 66.7% (22/33), 31.3% (5/16) and 12.5% (2/16), respectively.

These results indicate that the viability of the embryo is dynamic, and therefore time series analysis, i.e. using video, may not be well suited for embryo viability assessment because past viability is not necessarily a good predictor of future viability or pregnancy outcome. The viability of the embryo immediately prior to transfer, from a single static image, is a reliable predictor of viability. This is consistent with the current clinical practice of using Gardner score end-point assessment for embryo quality.

Results also suggest significant benefits from using time-lapse with AI, where AI continually assesses embryo viability over time using static images. The time point at which the embryo should be transferred to maximize pregnancy outcome is when the embryo has the greatest AI viability score.

Limitations, reasons for caution: Although evidence suggests past embryo viability is a limited predictor of future pregnancy, a side-by-side comparison of video versus single static image AI assessment would further verify that the historical or change in embryo development or viability has minimal impact on embryo viability assessment at the time prior to transfer.

Wider implications of the findings: Time-lapse and AI can beneficially change the way embryos are assessed. Continual AI monitoring of embryos enables optimization of which embryo to transfer and when, to ultimately improve pregnancy outcomes for patients. The findings also suggest that static end-point AI assessment is sufficient for predicting embryo implantation potential.

Trial registration number: not applicable

P-203 Applying artificial intelligence for ploidy prediction: The concentration of IL-6 in spent culture medium, blastocyst morphological grade and embryo morphokinetics as variables under consideration

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Study question: Would it be possible to predict embryo ploidy by taking into account conventional morphological and morphokinetic parameters together with IL-6 concentration in spent culture medium?

Summary answer: Our artificial neural network (ANN) trained with blastocyst morphology, embryo morphokinetics and IL-6 concentration distinguished between euploid/aneuploid embryos in 65% of the testing dataset.

What is known already: The analysis of spent embryo culture media represents the protein and metabolic state of the embryo and could be a non-invasive method of obtaining information about embryo quality. The impact of the presence/absence of several proteins in embryo culture samples over clinical results has been widely studied. The IL-6 is one of the most mentioned protein for its effect on embryo development, implantation and likelihood of achieving a live birth. In this initial attempt, we examined the predictive value for euploidy of a model that took into account the concentration of IL-6 in the spent culture medium.

Study design, size, duration: This prospective study included 319 embryos with PGT-A results. Out of the total, 127 were euploid and 192 aneuploid embryos. Concentration of IL-6 in spent embryo culture media (collected on the day of trophectoderm biopsy-fifth/sixth day of development), morphokinetic parameters (division time to 2 cells-t2; to 3 cells-t3, to 4 cells-t4; to 5 cells-t5 and time of blastocyst formation-tB) and blastocyst morphological grade (according to ASEBIR criteria) were considered to predict the embryo ploidy.

Participants/materials, setting, methods: Embryos were cultured in EmbryoScope. The chromosome analysis was performed using next-generation sequence technology. The concentration of IL-6 was measured in 20µL of spent embryo culture media with ELISA kits. Morphokinetic parameters were automatically annotated and the blastocyst morphology was evaluated by senior embryologists based on blastocele expansion, inner cell mass and trophectoderm quality. All the embryos were divided into 70% for training, 15% for validating and 15% for testing our ANN model with MatLab®.

Main results and the role of chance: The general description for the euploid embryo population was the following: 2% of the embryos were graded as A, 71% were graded as B and 28% were graded as C; the means and standard deviations were 25.32±2.97 hours (h) for t2, 35.33±5.15h for t3, 37.30±5.43h for t4, 48.24±6.62h for t5 and 103.93±12.8h for tB; and the average of IL-6 concentration was 1.51±0.70 pg/ml. The general description for the aneuploid embryo population was the following: 1% of the embryos were graded as A, 48% were graded as B and 51% were graded as C; the means and standard deviations were 26.13±3.51h for t2, 36.70±4.29h for t3, 38.20±4.24h for t4, 49.86±6.89h for t5 and 107.10±8.29h for tB; and the average of IL-6 concentration was 1.47±0.71 pg/ml. Our ANN model showed a higher general success rate as we increased the variables considered in the final prediction of euploid embryos. The accuracy, sensitivity and specificity for the testing dataset were: 0.60, 0.12 and 0.87 with morphokinetic parameters; 0.63, 0.24 and 0.93 with morphokinetics and IL-6 concentration; and 0.65, 0.16 and 0.96 with morphokinetics, IL-6 concentration and blastocyst morphological grade.

Limitations, reasons for caution: The low sensitivity and high specificity achieved in our models indicated that they were more capable of detecting aneuploid than euploid embryos. As this was a preliminary study, the small number of embryos included in the test (n=48) was also a limitation.

Wider implications of the findings: The results showed that our model tended to classify the embryos as aneuploid. More euploid embryos would be necessary to train our model and achieve better results in the prediction of chromosomally normal embryos. Further studies with large number of embryos and additional variables could improve the non-invasive ploidy prediction.

Trial registration number: not applicable

P-204 Dynamics of cavitation as a potential non-invasive predictor of mammalian blastocyst quality

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Study question: We wished to investigate whether dynamics of cavity formation can be used in embryo quality assessment.

Summary answer: Dynamics of mouse embryo cavitation reflects to certain extent blastocysts' developmental capabilities. It can be potentially used as a biomarker of mammalian embryo quality.

What is known already: During cavity expansion blastocyst pulsates, i.e. changes its volume in an oscillatory way. Recent studies performed on a mouse model have shown, that dynamics of cavitation, biomechanical properties of the

trophectoderm (TE) and embryo size are intertwined. Presence or absence of blastocyst contractions has been linked to particular parameters related to positive outcome of the in vitro fertilization procedures, but the data on influence of contractions on human embryos' developmental capabilities is often contradictory. Moreover, mostly in those studies only strong contractions (leading to a high volume loss) have been taken into consideration.

Study design, size, duration: We tested how postovulatory (in vitro or in vivo) or maternal aging of mouse oocytes affects dynamics of cavity formation and expansion in the resulting embryos (n=27, n=26 and n=30, respectively). Furthermore, we also analyzed almost 100 mouse blastocysts in order to correlate dynamics of their cavitation with their ability to form correct outgrowths (in vitro model of implantation).

Participants/materials, setting, methods: Mouse oocytes subjected to postovulatory (either in vivo or in vitro) or maternal aging were fertilized in vitro. Dynamics of cavity formation and expansion was assessed by time-lapse imaging; equatorial images were taken every 10 minutes. Blastocyst area was measured over time and compared to the outcome from control embryos. In another set of experiments, after the filming mouse blastocysts were cultured for additional 4 days to test their ability to form outgrowths.

Main results and the role of chance: We noticed, that mouse embryos which represent limited developmental potential (obtained from either postovulatory or maternally aged oocytes) and blastocysts developed from freshly fertilized young females' oocytes differ in terms of some parameters related to dynamics of cavitation, e.g. time of the initiation of cavity formation, frequency of contractions or mean loss of blastocyst's area during contraction. We observed that embryos obtained from oocytes subjected to maternal or postovulatory aging have distinct dynamics of cavitation. Moreover, we noticed slightly different effect on particular parameters related to cavitation between in vivo and in vitro version of postovulatory ageing. We also showed that blastocysts, which are unable to create proper outgrowths (i.e. too small or without epiblast cells), differ from embryos that differentiate into correct outgrowths in terms of certain parameters of cavitation dynamics. Our data indicates, that dynamics of cavity formation and expansion might be related to developmental potential of mouse embryo.

Limitations, reasons for caution: Further studies with extended group size and testing embryos' ability to implant in vivo are required to confirm our results. Moreover, we examined dynamics of cavitation only in a mouse model, so additional studies performed on other mammalian species are needed.

Wider implications of the findings: Our data proves, that dynamics of embryo cavitation reflects, to certain extent, developmental capabilities of mouse blastocysts. Therefore, it is possible that it can be a biomarker of embryo quality (in combination with parameters provided by other methods or solely) of other mammalian species, including humans.

Trial registration number: not applicable

P-205 Epothilone D as an actin cytoskeleton stabilizer improved mitochondria bioenergenesis and blastocyst formation of mouse preimplantation embryo

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Study question: What is primary factor of bioenergetics product activity between microtubule instability and the functional activity of mitochondria in embryo?

Summary answer: The actin cytoskeleton instability is presumably the primary cause for the bioenergenesis of mitochondrial function to the preimplantation embryo development.

What is known already: Mitochondria are cellular organelles dynamically moving and morphological changes. It provides for homeostatic energy to the cell. The dynamic property of the mitochondria is associated with the microtubule network in the cell. However, the stability of the microtubule was clearly identified for preimplantation embryo development.

Study design, size, duration: This study is designed to assess the ATP productivity of the mitochondria, and specifically to observe what its primary factor is in terms of providing microtubule stability in mammalian cells. Additionally, we investigated the relationship between blastocyst formation and actin cytoskeleton stabilization by EpD with 2-cell mice.

Participants/materials, setting, methods: We prepared the microtubule stability regulation model with the HEK293 cell line by using the microtubule stabilizer as an Epothilone D (EpD). Then we analyzed the metabolic activity of the cells through oxidative phosphorylation (OXP) ratios analysis. Also, we performed confocal live imaging to observe mitochondria morphology depending on the cells' microtubule. Next, we treated EpD to 2-cell culture media for the analysis of blastocyst development ratios.

Main results and the role of chance: EpD significantly increased fusion form. Also, EpD enhance bioenergy ratios like OXP in the mitochondria and functional activity related marker, like mTOR compared with the control. These results suggest that microtubule stabilization enhances mitochondrial metabolism by increasing oxygen consumption. Also, EpD in 2-cell culture media led to a significant increase in the speed of development and 50% higher hatched out blastocyst formation ratios compared to the control group.

Limitations, reasons for caution: This study had limited animal experiments. For the next study, we are planning with an aim to improve the quality and development ratios of human embryos by EpD.

Wider implications of the findings: Microtubule stabilizer has a possibility to recover the mitochondria's functional activity in the preimplantation embryo development. Mitochondrial functional activity along the actin cytoskeleton may play a pivotal role in determining the embryo quality and development ratios for archive pregnancy.

Trial registration number: non-clinical trials

P-206 Does oocyte vitrifcation affect morphokinetics of subsequent embryo development?

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Study question: Are the morphokinetic profiles, as assessed using time-lapse technology, of human embryos developed from vitrified oocytes different to those from fresh oocytes.

Summary answer: Vitrification of oocytes does have an effect on early developmental morphokinetic profiles, but this is normalized by the time the embryo has reached blastocyst.

What is known already: Vitrification of oocytes is now commonplace, but little is known about the effect this may have on subsequent embryo development.

Study design, size, duration: This was a retrospective data analysis, from 8 fertility clinics in the UK between 2012 and 2019. Embryos from patients in the vitrified group (n=557) were matched to fresh patient controls (n=539). The matching was performed based on the following criteria: type of treatment, patient age, cause of infertility and number of embryos.

Participants/materials, setting, methods: The embryos in each group were compared for mean morphokinetics of key developmental stages in hours post insemination (hpi). Parameters compared included early cleavage divisions (t2-t8), time to start of compaction (tSC), time to morula (tM), time to start blastulation (tSB), time to full blastocyst (tB) and duration of compaction (tB-tSC). Treatment outcomes were compared between the two groups, including percentage of blastocyst formation, clinical pregnancy rate, implantation rate and live birth rate.

Main results and the role of chance: The results showed a significant delay across all early cleavage divisions as follows for vitrified and fresh oocytes respectively: 2-cell (28.14 vs 26.10 ($p<0.001$)), 3 cell (37.56 vs 35.37 ($p<0.001$)), 4 cell (40.58 vs 37.54 ($p<0.001$)), 5 cell (50.31 vs 47.14 ($p<0.001$)), 6 cell (53.99 vs 50.87 ($p<0.001$)), 7 cell (57.08 vs 54.48 ($p<0.001$)) and 8 cell (61.26 vs 58.91 ($p<0.01$)). In addition, tSC was also significantly delayed in the vitrified group (80.65 vs 76.36 ($p<0.001$)). However, the compaction stage was significantly shorter in the vitrified oocytes (19.02 vs 22.45 ($p<0.001$)). Therefore, there was no difference in the time that embryos derived from fresh and vitrified

oocytes reached the blastocyst stage (108.03 vs 107.78 (p>0.05)). No difference was found in clinical pregnancy, implantation or live birth rates but significantly fewer blastocyst developed from vitrified oocytes compared to fresh (36.09% vs 42.4% (p<0.05)).
Limitations, reasons for caution: Although this was a matched analysis, it was a retrospective in nature therefore is subject to confounders. However, it would be problematic to perform a prospective randomized controlled trial to address this study question given the need to randomize patients to elective freezing of oocytes prior to embryo creation.
Wider implications of the findings: Vitrification of oocytes may affect early developmental morphokinetic profiles, but any effect is normalized by the time the embryo has reached blastocyst. However, fewer blastocysts may develop following oocyte vitrification. This may have implications for oocyte donation banks and those patients choosing to cryopreserve oocytes.
Trial registration number: na

P-207 Heterogoneic cell division proven to occur in bovine zygotes

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Study question: We hypothesize that the zygote can segregate parental genomes via a non-canonical pathway. We coined this heterogoneic cell division. Can we proof the existence of this new segregational pathway?
Summary answer: We confirmed the existence of this non-canonical segregation mechanism leading to mixoploidy and provide a catalogue of abnormal zygotic divisions.
What is known already: Embryos show a high degree of chromosomal instability leading to chromosomal mosaicism. Chromosomal aberrations affect the developmental potential. We developed haplarithmisis which determines the single-cell genome-wide haplotype and copy number and allows to deduce the parental and segregational origin. Analysis of cleavage-stage bovine embryos by haplarithmisis, discovered the presence of uniparental and biparental blastomere lineages in individual embryos. We hypothesized that whole genome segregations can occur via a non-canonical zygotic division, a process termed "heterogoneic" division. Abnormal zygotic division has been observed in bovine and human *in vitro* produced zygotes using time-lapse, but parental genome segregation has never been disclosed.
Study design, size, duration: We hypothesized that abnormal cytokinetics and spindle mechanics may underlie the segregation of parental genomes in a separate blastomere line. *In vitro* produced zygotes were monitored by time-lapse microscopy. Zygotes cleaving 3 or 4 cells were disaggregated, picked and analysed by haplarithmisis.
Participants/materials, setting, methods: Blastomeres from bovine *in vitro* produced zygotes cleaving directly into 3 or 4 blastomeres, identified by time-lapse monitoring, were tubed following zona removal and blastomere dissociation and whole-genome amplified. Samples were subsequently hybridized on Illumina Bovine HD BeadChip SNP arrays. Data was analyzed by haplarithmisis, using a the siCHILD-bovine algorithm, to infer the haplotypes and the copy number of the parental genomes. Blastomeres showing failed haplarithmisis plots were low-coverage whole-genome sequenced on a HiSeq4000 sequencer. Main results and the role of chance: We obtained 25 bovine embryos, comprising 82 blastomeres, derived from 12 families (12 cows and 2 bulls) that cleaved directly into 3 or 4 blastomeres. Sixteen, 7 and 2 out of 25 zygotes cleaved respectively in 3 cells, 4 cells and 3 cells and a fragment. In at least 20 embryos, more than one paternal haplotype was identified, showing that a polyspermic fertilization resulted in an abnormal division. All embryos contained a whole-genome abnormality in at least one blastomere, resulting in mixoploid (5), mixed diploid biparental and androgenetic (12), polyploid (4), mixed gynogenetic and androgenetic (2) and androgenetic (2) embryos. Twenty-one embryos had at least one blastomere containing a uniparental signature. Based on the blastomere haplotype profiles we classified the embryos in six segregation categories. In twelve blastomeres haplarithmisis failed. Massive parallel sequencing of the amplified DNA showed the presence of mitochondrial DNA, indicating the blastomere did not contain any genomic DNA. This observation confirms that heterogoneic cell division does occur via different non-canonical zygotic segregations, which result in a variety of chimeric and mixoploid embryos constitutions.
Limitations, reasons for caution: These findings apply to a small set of bovine *in vitro* produced abnormally cleaving embryos. Segregation patterns may be incomplete and their true *in vitro* and *in vivo* prevalence remains unknown. Based on the haplotypes the non-canonical divisions have been reconstructed. Those patterns can now be evaluated and tested.
Wider implications of the findings: This study shows that heterogoneic cell division occurs. We hypothesize this non-canonical division occurs frequently in both *in vitro* and *in vivo,* not only in cattle but also in man and hypothesize that persistence of such cell lines might explain the development of androgenetic tumorous outgrowths and mosaic uniparental individuals.
Trial registration number: not applicable

P-208 Oleic acid rescues altered autophagy induced by palmitic acid during mouse preimplantation development

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Study question: The aim of the study is to identify the autophagic profile and the effects of fatty acid treatments on autophagic activity in preimplantation mouse embryos.
Summary answer: Autophagic activity varies significantly in early stages of mouse preimplantation development; exposure to fatty acids alters the embryonic autophagy profile.
What is known already: Obesity is one of the top comorbidities for infertility, and obese individuals have elevated fatty acid levels. In serum, palmitic acid (PA) and oleic acid (OA) are the most abundant saturated and unsaturated fatty acids, respectively. We recently reported that PA impairs blastocyst development, affects mitochondrial reactive oxygen species, triacylglycerol levels, and endoplasmic reticulum stress pathways during mouse preimplantation development. Interestingly, the addition of OA counteracts those effects. Autophagy plays an essential role in embryo development, as knock-out of a key autophagy protein is embryonic lethal. Little is known about the autophagic profile in fatty acid treated mouse preimplantation embryos.
Study design, size, duration: Pools of 20 – 25 mouse embryos were collected from gonadotrophin super-ovulated and mated CD1 female mice. Two-cell stage embryos were treated with 100 μM PA and 250 μM OA, alone and in combination, and 1.5% bovine serum albumin media (control) within KSOMaa media for 18, 24, and 48 hours *in vitro*. The detection of various autophagic markers were evaluated by immunofluorescence microscopy and RT-qPCR.
Participants/materials, setting, methods: mRNA levels of autophagic markers were measured using RT-qPCR with the Taqman primers and Universal PCR Mix. Immunofluorescence staining of LC3 puncta (marker for autophagosome formation) was performed using LC3A/B polyclonal antibody (Invitrogen PA1-16931) and DAPI (4 ,6-Diamidino-2-phenylindole dihydrochloride) was used to stain for cell nuclei. Analysis of LC3 puncta was performed using ImageJ software. Images were acquired using an LSM 800 laser scanning confocal microscope. Data analysis was completed by GraphPad Prism software.
Main results and the role of chance: Mouse preimplantation embryos showed no change in mRNA levels of autophagic markers (*Bcln1, ATG3, ATG5, and LC3*) relative to the control group after 48-hours exposure of 100 μM PA and 250 μM OA treatments, alone and in combination.

The number of LC3 puncta was measured and analyzed as a reflection of autophagic activity in mouse preimplantation embryos. Under the fatty acid-free condition, the average number of LC3 puncta per blastomere was significantly decreased after 18 hours of development ($p < 0.005$). However, the average number of LC3 puncta per blastomere at 18, 24, and 48 hours were not significantly different from each other ($p = 0.2724$).

Following 100 μM PA and 250 μM OA treatments, alone and in combination, autophagic activity was impacted by the presence of fatty acids. Mouse

preimplantation embryos exposed to control and fatty acid treatment groups demonstrated no significant differences in LC3 puncta per blastomere at 18- and 24-hours treatment time ($p = 0.5381$; $p = 0.7829$). However, embryos exposed to 48 hours of PA treatment had a significantly greater number of LC3 puncta per blastomere than embryos exposed to 48 hours of OA and PA and OA combination treatments ($p < 0.05$).

Limitations, reasons for caution: Although LC3 puncta count (autophagosome formation) is impacted by fatty acid treatment, autophagic flux must be measured to fully investigate autophagic activity during mouse preimplantation development. These processes need to be measured in human embryos cultured *in vitro*.

Wider implications of the findings: Profiling autophagic activity in fatty acid treated mouse preimplantation embryos would guide future investigations on pharmacological modulation of autophagy as a therapeutic intervention for developmentally delayed embryos. With the information gained, we aim to develop strategies to assist overweight and obese patients with their fertility needs.

Trial registration number: not applicable

P-209 Artificial collapse of human expanded blastocysts protects the quality of embryos during vitrification/warming procedure

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Study question: Does artificial collapse of human blastocysts before vitrification affect the post warming quality of embryos and/or the reproductive outcome?

Summary answer: This study suggests that artificial collapse with laser pulse before vitrification significantly benefits blastocyst quality post-warming without improving reproductive outcome.

What is known already: The efficiency of vitrification of blastocysts can be influenced by various factors, such as the stage of the expansion and the quality of the embryos. Expanded blastocysts seem to be more sensitive and prone to cryo-injury during vitrification because of the large amount of blastocoelic fluid which may cause insufficient dehydration. Artificial collapse (AC) with micro-needles or with laser pulse can improve the vitrification procedure and protect the expanded blastocysts from cryoinjuries by reducing the fluid of the blastocoel.

Study design, size, duration: This prospective randomized study was performed at Embryolab Fertility Clinic, in Thessaloniki, Greece between July 2020 and November 2020 and included 94 ICSI treatments with no fresh embryo transfer. Patients with more than 4 blastocysts were randomized and allocated to the study (AC) group or control group. Randomization list was created by a computer-based program. The embryos were vitrified at the blastocyst stage and the best 1-2 embryos were transferred in a subsequent cycle.

Participants/materials, setting, methods: Patients were divided in two groups: AC group (n=46) where 1 or 2 best expanded blastocysts were artificially collapsed by a laser pulse before vitrification and control group (n=48) where the corresponding best expanded embryos remained untreated before vitrification. The embryos were graded according to Gardner's criteria and vitrified using open system. Quality of embryos, pregnancy rate and clinical pregnancy rate from the first warming cycle were the parameters that were analyzed using z-test.

Main results and the role of chance: The analyzed embryos were in total 171, 85 in the AC group and 86 in the control group. The day 5 embryos in each group were 6.89 (±3.41) and 6.50 (±3.38) and the number of embryos per embryo transfer was 1.85 (±0.36) and 1.79 (±0.41) respectively. Cryoinjury was determined as the presence of degenerated cell in ICM or TE. In the AC group cryo-injury was significantly lower than in the control group [31.11% with at least 1 embryo per embryo transfer and 52.08% respectively ($p=0.0356$), 6.67% with cryo-injury in both embryos and 14.58% respectively ($p<0.0001$), 8.89% with cryo-injury in ICM and 10.42% respectively ($p<0.0001$), 15.56% with cryo-injury in trophectoderm and 27.08% respectively ($p<0.0001$), and 6.67% with cryo-injury in both ICM and trophectoderm and 12.50% respectively ($p<0.0001$)]. Pregnancy rate (positive b-hcg) was not significantly different between the two groups (62.22% in AC group and 76.09% in control group, $p=0.1479$), nor was the clinical pregnancy rate (52.38% and 60.87% in AC and control group respectively, $p=0.4208$).

Limitations, reasons for caution: The number of cases included in this study was limited and the live birth rate was not yet available. More prospective randomized studies are needed in order to validate the benefit of artificial collapse before the vitrification procedure.

Wider implications of the findings: Laser artificial Collapse does not compromise expanded blastocyst quality, on the contrary it seems to reduce the amount of cryoinjury observed post warming. Whether it can improve the reproductive outcome, remains to be examined in larger scale studies.

Trial registration number: not applicable

P-210 Abnormal cleavage patterns during embryo preimplantation development and their effect on blastulation: an overview from IVF patients with multiple IVF cycles in a time-lapse incubator.

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Study question: How common abnormal cleavage patterns (ACP) are in IVF and what are their consequences on embryo developmental competence?

Summary answer: ACP might affect up to 25% of the 2PN-zygotes, independently from patients'/cycles' characteristics, and mostly cause embryo developmental arrest around the 4-to-8-cell transition.

What is known already: Since its implementation in IVF, time-lapse-microscopy (TLM) allowed the standardization of embryo culture within undisturbed incubators, but it has not improved embryo selection especially if blastocyst transfer is performed. Nevertheless, TLM holds the potential for boosting our knowledge of embryo preimplantation development. In particular, a continuous observation of embryo morpho-dynamics unveiled peculiar blastomere cleavage patterns previously unidentifiable with a static morphological assessment. These events are possibly associated with massive mitotic errors, affecting both chromosomes and cytoskeletal components, as well as downstream metabolic imbalances. Still, the causes of ACP and their consequences on embryo developmental/reproductive competence require further investigation.

Study design, size, duration: Observational study including 75 patients (age:38.6±3.7yr, FSH:8.8±3.6IU/l, AMH:1.7±1.3ng/ml; BMI:21.4±2.4) who conducted multiple IVF cycles (N=160; 8.7±5.0 cumulus-oocyte-complexes and 6.3±3.6 metaphase-II collected; 201±245 days between first and second cycles) in a time-lapse incubator between 2014-2020. All annotations were performed blindly by two operators and confirmed by a third in case of discordance. The outcomes were the blastulation rate after any ACP, their association between each other and with patients'/cycles' characteristics.

Participants/materials, setting, methods: We included only ICSI-cycles after ovarian-stimulation with blastocyst culture conducted in the Embryoscope. Overall, 981 metaphase-II were inseminated and 677 2PN-zygotes annotated. The ACP investigated were: (i)cytokinesis-failure, formation of cytoplasmic septa without cell division; (ii)Chaotic-cleavage, disordered and uneven cleavages; (iii) Direct-unequal-cleavage (DUC), cleavage of zygotes or single blastomeres directly into 3; (iv)Rapid-cleavage, t3-t2<5hr; (v)Reverse-cleavage, fusion of 2 blastomeres into 1; (vi)Fragmentation, presence of numerous non-nucleated fragments; (vii)Blastomeres' exclusion/extrusion, nucleated cells excluded/extruded from the morula.

Main results and the role of chance: Among the 2PN-zygotes, the prevalence of cytokinesis-failure was 5.9% (N=40/677), 15.7% for chaotic-cleavage (N=106/677), 18.6% for DUC (N=126/677), 4.1% for rapid-cleavage (N=28/677), 3.5% for reverse-cleavage (N=24/677) and 24.1% for fragmentation (N=163/677). Among the morulae, the prevalence of blastomere exclusion/extrusion was 27% (N=109/410;1.5±1.2 excluded/extruded cells,range:1-7). The risk for reverse-cleavage was higher among 2PN-zygotes facing failed-cytokinesis (N=8/40,20% versus N=16/637,2.5%, OR:9.7,95%CI:3.9-24.3,$p<0.01$). Fragmentation was instead higher among 2PN-zygotes undergoing chaotic cleavage (N=47/106,44.3% versus N=116/571,20.3%, OR:3.1,95%CI:2-4.8,$p<0.01$) or DUC (N=46/126,36.5% versus N=117/551,21.2%, OR:2.1,95%CI:1.4-3.2,$p<0.01$). Lastly, higher prevalence of blastomeres' exclusion/extrusion were reported among morulae obtained after chaotic-cleavage (N=17/29,58.6%

versus N=92/381,24.1%, OR:4.4,95%CI:2-9.7,p<0.01), DUC (N=26/37,70.3% versus N=83/373,22.3%, OR:8.3,95%CI:3.9-17.4,p<0.01) and in presence of fragmentation (N=79/195,75.2% versus N=30/305,9.8%, OR:27.8,95%CI:15.6-49.8,p<0.01); only a higher trend after rapid-/reverse-cleavage.

No predictive factor of ACP was identified among patients' and cycles' characteristics, except for higher risks of fragmentation (OR:2.6,95%CI:1.1-6.3,p=0.04) and blastomeres' exclusion/extrusion (OR:2.7,95%CI:1.1-7.2,p=0.04) among patients with previous experience with these events.

The viable-blastocyst rate per 2PN-zygote was 45.1% (N=305/677). It was lower in case of failed-cytokinesis (N=12/40,30% versus N=293/637,46%, OR:0.5,95%CI:0.25-0.99,p=0.05), chaotic cleavage (N=20/106,18.9% versus N=285/571,49.9%, OR:0.23,95%CI:0.14-0.39,p<0.01), DUC (N=27/126,21.4% versus N=278/551,50.5%, OR:0.27,95%CI:0.17-0.42,p<0.01), rapid-cleavage (N=6/22,21.4% versus N=299/649,46.1%, OR:0.32,95%CI:0.13-0.8,p=0.02), and reverse-cleavage (N=5/19,20.8% versus N=300/653,45.9%, OR:0.31, 95%CI:0.11-0.84,p=0.02). No difference was instead shown in case of fragmentation and/or blastomeres' exclusion/extrusion.

Limitations, reasons for caution: The patients included were poor-prognosis women undergoing ≥2 cycles. We are expanding the sample size to account for all cycles conducted in time-lapse incubators. Larger sample size will provide also statistical-power to investigate the effect of ACP on blastocysts' chromosomal and implantation competence, and more visualizations of rapid-/reverse-cleavage events.

Wider implications of the findings: After ACP,developmental-arrest mostly occurs around the 4-to-8-cell transition (50-70% versus ~30%), when embryonic-genome-activation takes place. Surviving embryos often fragment and/or exclude/extrude blastomeres at morulation, without further impact on blastulation-rates. Moreover, ACP seem independent from patients'/cycles' characteristics. These evidence incite future Research on the biological/genetic mechanisms triggering ACP and their consequences.

Trial registration number: None

P-211 Double warming and double vitrification for euploid embryos does not affect implantation nor ongoing pregnancy rate

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Study question: Does exposure of embryos to double vitrification and double warming affect the chances of ongoing pregnancy for patients undergoing PGT-A and transfer euploid embryos?

Summary answer: Our analysis shows that there is no statistically significant difference in implantation or ongoing pregnancy rate between single or double vitrification/warming cycles.

What is known already: The use of PGT-A is increasing in the last years and progressively more patients opting in for this, in order to reduce time to pregnancy. Implantation failures prior to genetic testing or the incidence of no-result embryos post PGT-A are some of the scenarios that expose the embryos to multiple rounds of vitrification/warming cycles. The exact effect that such exposure has on embryos is still to be investigated and confirmed as to whether it affects the outcome (i.e. implantation/ongoing pregnancy rate) or the future health of the child.

Study design, size, duration: Our analysis is a retrospective observation study of data collected from 151 consecutive frozen euploid embryo transfers (FET). These were performed at a single centre between January-December 2020. Two groups were created for this study. The first group includes euploid embryos that were transferred post being exposed to single vitrification/warming (n=126). In the second group euploid embryos were exposed twice to vitrification/warming (n=25). Statistical analysis using chi-square test and statistical significance was calculated when p≤0.05.

Participants/materials, setting, methods: Blastocysts from 151 patients were split into two groups based on the number of vitrification/warming cycles that they underwent prior to FET. The first group includes embryos that were subjected to trophectoderm biopsy and were then vitrified (n= 126). The second group includes embryos that were initially vitrified without undergoing PGT-A analysis. Following implantation failures, their remaining embryos were warmed, biopsied and re-vitrified. Post PGT-A analysis euploid embryos were then re-warmed and transferred (n= 25).

Main results and the role of chance: For the first group (A), 450 blastocysts (day 5-7) were subjected to trophectoderm biopsy, where 5-cells taken, and embryos were then vitrified. Post PGT-A analysis 260 euploid embryos identified. From them 126 embryos transferred in frozen replacement cycles, where the mean embryo age for the group was 36.1±4.2. The grade of embryos transferred were of 4BC or better based on Gardner's grading system. The implantation and ongoing pregnancy rate for this group was 62%.

For the second group (B), 101 blastocysts (day 5-7) warmed, in order to undergo trophectoderm biopsy and were then re-vitrified. Post PGT-A analysis 49 euploid embryos identified. From them, 25 embryos transferred in frozen replacement cycles, where the mean maternal age for the group was 35.05±5.2. The grade of embryos transferred were of similar quality to group A. The implantation and ongoing pregnancy rate for this group was 64%.

Statistical analysis confirmed that there is no statistical difference between the groups (p=0.74).

In addition, 60% of patients (n=5) who had double vitrification, double biopsy and double warming have ongoing pregnancy.

In conclusion, for transferrable quality euploid blastocysts, double vitrification has comparable reproductive outcomes as in single vitrification, thereby supporting the efficacy of double vitrification/warming when necessary.

Limitations, reasons for caution: This study uses a small sample size of patients. The data are observational and were retrospectively analysed so unknown confounders could not be assessed. The addition of more cycles and further multivariate analysis, including the child's health is essential for confirmation of the findings. However, initial results are very reassuring.

Wider implications of the findings: Our study has implications for clinical practice and patient counselling. Especially in patients that they choose to undergo PGT-A with pre-vitrified embryos post implantation failures with non PGT-A tested embryos.

Trial registration number: N/A

P-212 Mitochondrial DNA content shows a significant association with timing of human embryo development and fertility diagnosis in euploid embryos

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Study question: Does mitochondrial DNA content (mtDNA) correlate with clinical parameters and embryo morphokinetics using advanced time-lapse technology?

Summary answer: mtDNA correlated with embryo morphokinetics and the growth trajectory of euploid embryos. Maternal age, anti-mullerian hormone level and fertility diagnosis were significantly associated with mtDNA.

What is known already: With the push towards single embryo transfers, laboratories are working to improve embryo selection. In addition to conventional microscopy, preimplantation genetic testing and time-lapse microscopy have been utilized to aid in embryo selection. More recently, as mtDNA may represent the energy potential of an embryo, some data have supported the use of mtDNA as an additional tool. Limited studies have suggested that a lower amount of mtDNA is associated with higher rates of implantation and improved embryo quality.

Study design, size, duration: This is a retrospective chart review. All embryos that underwent preimplantation genetic testing for aneuploidy (PGT-A) between January to December of 2020 were studied.

Participants/materials, setting, methods: Women undergoing in vitro fertilization (IVF) with intracytoplasmic sperm injection undergoing PGT-A were studied. All patients were from a single academic institution. This study exclusively examined the characteristics of euploid embryos. Mitochondrial DNA content was expressed as a ratio of mtDNA:nDNA (MitoScore). Time-lapse imaging was utilized to evaluate embryo development every 15 minutes in 5-7 focal planes. Chi square test and Spearman correlation analysis were performed with a p-value of <0.05 considered significant.

Main results and the role of chance: A total of 494 embryos from 52 women who underwent 58 IVF cycles were cultured to blastocyst and 331 embryos were biopsied for PGT-A evaluation. Of these, 132 embryos were diagnosed as euploid. A moderate positive correlation was found between MitoScore and time to morula, time to blast and time to expanded blast (correlation value 0.54, 0.50 and 0.54, respectively; p<0.001). Consistent with this trend, day 5 blastocysts had a significantly lower MitoScore values than day 6

blastocysts (20.2 v. 29.2; p<0.001). When examining all biopsied euploid embryos, no significant association was found between MitoScore, blastocyst maturity, trophectoderm or inner cell mass scores.

Our data also demonstrated a positive correlation between MitoScore and maternal age (correlation factor 0.33; p<0.001). A negative association between MitoScore and serum anti-mullerian hormone levels (correlation factor -0.20; p<0.021) was also noted. Of particular interest was the significant association between fertility diagnosis and mitochondrial score (p<0.001).

Even amongst euploid embryos, mtDNA content varied widely, potentially reflecting differences in embryo potential and quality. Additionally, the significant difference in MitoScore between that day 5 and day 6 blastocysts may reflect a fundamental difference in cytoplasmic characteristics and requires further study.

Limitations, reasons for caution: Due to the study cohort of euploid embryos undergoing PGT-A, this study was biased for the selection of high grade embryos. This limited diversity in embryo quality may have masked other potential associations between mitochondrial content and blastocyst quality.

Wider implications of the findings: mtDNA may be additional tool aiding in embryo selection as IVF labs work to improve pregnancy rates while minimizing the risks of transferring multiple embryos. To our knowledge, this is the largest study assessing the relationship of mtDNA content of blastocysts and the timing of embryo development using time-lapse imaging.

Trial registration number: None

P-213 Comparative optical analysis of spindle reformation after oocyte vitrification with media containing differing basal formulations

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Study question: Do the base ingredients and total composition of vitrification media have an effect on meiotic spindle reformation in warmed donor oocytes?

Summary answer: Meiotic spindle reformation occurs more readily in donor oocytes vitrified and warmed with a contemporary culture media based vitrification formulation than in traditional vitrification media.

What is known already: Embryo culture trends continue towards more freeze-all cycles and oocyte preservation is becoming more prevalent across all age groups, thus vitrification continues to serve a pivotal role in today's laboratory; however, the formulation of vitrification media remains largely unchanged from the m199 or mHTF base composition of antiquated slow freeze media. Literature identifies temperature as a key determiner in spindle reformation. Our preliminary data utilizing a robust vitrification medium with a basal formulation specifically designed for human embryo growth demonstrates a pronounced positive effect on oocytes post-warm in regard to spindle recovery time.

Study design, size, duration: In a prospective study, 30 oocytes obtained from a diverse population of 10 oocyte donors donating to an egg bank were imaged prior to vitrification to identify the meiotic spindle using the Oosight Imaging System (Hamilton Thorne). Donor oocytes were then split between two vitrification media groups, Vitrification Kit NX (FujiFilm Irvine Scientific) and Vitrification Media (Kitazato USA). After warming, oocytes were again imaged at various time points and meiotic spindle retardance was noted.

Participants/materials, setting, methods: Oocytes from approved egg bank donors were imaged, vitrified and warmed using protocols recommended by the manufacturers. The oocyte donors were all cycled and retrieved in the same private IVF Clinic and Laboratory between October 2019 and February 2020. Temperature of media, culture dishes, incubators and work stations were all monitored and maintained. Retrieved oocytes were imaged prior to vitrification. Oocytes were imaged immediately upon warm, and at 1 and 3 hours post warm.

Main results and the role of chance: Oocyte survival was similar across the two media groups, with Vitrification Kit NX at 86.7% and Vitrification Media at 80.0% survival. 100% of the degenerated oocytes in Vitrification Kit NX did not display an initial meiotic spindle when imaged prior to vitrification as opposed to 67% of the degenerate oocytes in Vitrification Media. Upon warming, immediate imaging showed oocytes from Vitrification Kit NX displayed a spindle at a rate of 41.7%, while Vitrification Media allowed this in 0% of the oocytes. At 1-hour post warm, Vitrification Kit NX and Vitrification Media displayed meiotic spindles in 83.3% and 22.2% of oocytes, respectively. The final time point was imaged at 3-hours post warm and showed meiotic spindle reformation in 91.7% of Vitrification Kit NX oocytes and 66.7% of Vitrification Media oocytes. While none of the time points imaged show significance, there is a defined trend towards a faster rate of meiotic spindle recovery in vitrification media formulated with a more modern culture media base.

Limitations, reasons for caution: The preliminary findings of this study offer that the composition and formulation of the vitrification medium perhaps do have an effect on the reformation of the meiotic spindle post-warm; however, more information is needed and a larger population size must be examined prior to discerning any significance.

Wider implications of the findings: If, through further study and imaging, it is ascertained that meiotic spindle reformation is determined in part by the vitrification media composition, it could lead to potentially healthier warmed oocytes for the patient and less workload and scheduling stress on the embryologists due to shorter wait times prior to ICSI.

Trial registration number: not applicable

P-214 Effect of artificial activation of oocytes (AOA-ICSI) on the ploidy status of the resultant blastocysts. A sibling-oocytes pilot study

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Study question: Will artificial activation of oocytes alter the ploidy status of the resultant blastocysts? A sibling-oocytes pilot study

Summary answer: AOA-ICSI does not increase the risk of having aneuploidy blastocysts and can improve the fertilization rate in patients with sperm factor deficiency.

What is known already: Despite introducing ICSI as an aid to improve chances of fertilization, fertilization failure can still occur in 2-3% of ICSI cycles. Fertilization is a complex process triggered by a cascade of events following calcium (Ca2+) oscillations. Evidence suggests that the deficiency, localization or altered structure of the sperm-derived protein PLCζ in oocyte activation may be a reason for meiotic II arrest in the oocyte. Artificial oocyte activation has been proposed to compensate for the lack of calcium oscillation and resumes meiotic progression. There are however insufficient studies to determine its effect on the chromosomal status of the resultant blastocysts.

Study design, size, duration: This is a prospective, randomized study conducted at our Center from August-October 2020. A total of 20 couples intended for ICSI + Preimplatation Genetic Testing for Aneuploidy (PGT-A) cycles were recruited based on fulfilling one of the following criteria: 1) previous total fertilization failure (TFF), 2) history of low fertilization rate (<30%), 3) more than 2 cycles of failed IVF cycles (no implantation) 4) poor embryo development (no blastocysts formed) and 5) severe male factor.

Participants/materials, setting, methods: A total of 231 MII oocytes underwent randomization in a 1:1 ratio between AOA-ICSI and control group. All oocytes are subjected to ICSI treatment. Oocytes in the AOA-ICSI group are treated in 25µl droplets 10µM ready to use bicarbonate buffered calcium ionophore (Kitazato, Japan) for 15 minutes post-ICSI. The blastocysts were biopsied and subjected to PGT-A. Primary outcome was the aneuploidy rate and secondary outcomes were fertilization rate and blastocyst rate.

Main results and the role of chance: There were 11 out of 40 (27.5%) aneuploid blastocysts in the AOA-ICSI group and 7 out of 23 aneuploid blastocysts (30.4%) in the control group [odds ratio (OR) = 0.87; 95% confidence interval (CI) 0.28-2.68, p=0.8040). There was no statistically significant difference between both groups. However, fertilization rate of the AOA- ICSI group was significantly higher than the fertilization rate in the control group (68.6% vs 49.6% respectively, OR=2.22; 95% CI, 1.31-3.81, p=0.0034). There were 40 blastocysts formed in the AOA-ICSI group and 23 blastocysts formed in the control group. It was found that the AOA-ICSI group yielded a higher blastocyst rate (49.4%) compared to the control group (41.1%) (OR = 1.40; 95% CI, 0.71 to 2.78, p=0.3379) but the difference was not statistically significant.

Limitations, reasons for caution: The possibility of TE cells biopsied may not be representative of the whole blastocyst makes it possible to have false clinical data. The dosage and time were also not evaluated in this study as

exposure time was found to be a critical factor of fertilization rate in a previous study.
Wider implications of the findings: This study showed that AOA-ICSI does not increase the risk of having aneuploidy blastocysts and can improve the fertilization rate in patients with sperm factor deficiency. Additional studies involving a larger number of patients with more specific indication can further justify the benefits of AOA as a therapeutic application.
Trial registration number: NA

P-215 The degree of perivitelline space (PS) at the pronuclear stage affects subsequent embryonic development in human zygotes

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Study question: Was embryonic development affected by the degree of perivitelline space (PS) at the pronuclear stage in human zygotes?
Summary answer: Zygotes with a fully surrounding PS showed less cytoplasmic fragmentation and a higher blastocyst development rate (BDR) than zygotes with a partially surrounding PS.
What is known already: We previously used abnormally-fertilized oocytes (zygotes with three pronuclei; 3PN), donated by ART patients in our clinic who gave written consent for the research. The zona pellucida (ZP) was artificially removed from these oocytes at the pronuclear stage, termed ZP-free culture. The resultant ZP-free 3PN embryos showed less cytoplasmic fragmentation and a higher rate of good-quality embryos (GQE) compared with ZP-intact embryos. Furthermore, in our clinical setting, the rate of GQE and BDR of normally-fertilized embryos were clearly improved by ZP-free culture in patients with recurrent failure of ART treatments due to severe cytoplasmic fragmentation at the early cleavage stage.
Study design, size, duration: This study included 49 patients who gave written informed consent for our study and were treated with ART in our clinic between March and December 2020. Embryonic development was compared between zygotes with a fully surrounding PS [PS(+)] with those with a partially surrounding PS [PS(-)] at the pronuclear stage. Furthermore, the ZP of PS(-) embryos were artificially removed at the pronuclear stage, and the rate of GQE and BDR were compared with ZP-intact embryos.
Participants/materials, setting, methods: The degree of PS in 128 zygotes was confirmed by hypertonic preparation using 0.125M sucrose-containing HEPES medium. PS(+) and PS(-) embryos were both cultured as ZP-intact, and the rate of GQE was compared. Furthermore, 223 zygotes were divided into three groups: 1) PS(-)/ZP-intact, 2) PS(-)/ZP-free, and 3) PS(+)/ZP-intact, and cultured in an incubator equipped with time-lapse monitoring up to Day 7, and the rate of GQE, BDR and useable embryos were compared between each groups.
Main results and the role of chance: The degree of PS was confirmed by a hypertonic preparation (shrinkage of the ooplasm) in 128 normally-fertilized zygotes obtained from 44 cases. There were 86 PS(-) (67.2%) and 42 PS(+) (32.8%) zygotes. The mean maternal age was 35.9 in PS(-) and 40.5 in PS(+) ($P < 0.01$), and the rate of GQE was significantly higher in PS(+) [64.3% (27/42)] than in PS(-)[38.4% (33/86)] ($P < 0.01$). In addition, of 223 normally-fertilized zygotes obtained from 41 cases, there were 51 PS(-)/ZP-intact (Group 1), 132 PS(-)/ZP-free (Group 2) and 40 PS(+)/ZP-intact (Group 3) zygotes. The rate of GQE was significantly lower in Group 1 [29.4% (15/51)] compared with Group 2 [59.8% (79/132)] and Group 3 [62.5% (25/40)] ($P < 0.01$). BDR was also significantly lower in Group 1 [51.3% (10/39)] compared with Group 2 [75.0% (99/132)] and Group 3 [65.0% (13/20) ($P < 0.01$).
Limitations, reasons for caution: Although the artificial removal of ZP at the pronuclear stage (ZP-free culture) clearly increased the rate of GQE, embryonic development was not improved in all cases. It seems that this procedure is only effective in embryos with a viable ooplasm.
Wider implications of the findings: The degree of PS at the pronuclear stage affects subsequent embryonic development in human zygotes. The artificial removal of ZP at the pronuclear stage (ZP-free culture) helps to suppress fragmentation and leads to an increase in GQE and BDR, and eventually, improves pregnancy rate in cases with severe fragmentation.
Trial registration number: non

P-216 Successful pregnancies and deliveries in patients with a recurrent failure of ART treatments following artificial removal of the zona pellucida (ZP) at the pronuclear stage

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Study question: Can a novel embryo culture method that artificially removes the ZP at the pronuclear stage yield successful pregnancy in patients with poor-quality embryos and/or blastocysts?
Summary answer: A blastocyst transfer after ZP-free culture can result in pregnancy for patients who cannot obtain good quality blastocysts from conventional culture methods.
What is known already: Perivitelline threads are been associated with the formation of cytoplasmic fragments. We had previously observed perivitelline threads in the adhesive region between the ooplasm and the ZP at the first cleavage in human embryos. We removed the ZP at the pronuclear stage in 71 abnormally fertilized oocytes (zygotes with three pronuclei), donated after conventional IVF (c-IVF), and termed them ZP-free 3PN. We found ZP-free 3PN embryos could be cultured without losing blastomere adhesions. Furthermore, the rate of good quality embryos was significantly higher in ZP-free 3PN embryos compared with ZP-intact embryos (ZP-intact 2PN/2PB and 3PN embryos; $P < 0.05$).
Study design, size, duration: This study was conducted in two cases selected among patients who underwent ART treatment in our clinic between 2018 and 2019. Cases were selected if they lacked good quality blastocysts in previous c-IVF/Intracytoplasmic Sperm Injection (ICSI) cycles due to massive cytoplasmic fragmentation at the first and second cleavage. We performed a clinical trial of ZP-free culture from December 2019 to March 2020.
Participants/materials, setting, methods: Two cases were selected for this trial. Normally fertilized oocytes were grouped as ZP-free or ZP-intact. For the ZP-free group, 2PN embryos were placed in 0.125M sucrose-containing HEPES to reduce ooplasm size, then ooplasms were completely separated from ZPs by a laser and pipetting. ZP-free and ZP-intact embryos were cultured with time-lapse imaging for up to seven days. Resultant blastocysts were either transferred into uterus or cryopreserved on Day5/6/7 for future embryo transfer cycles.
Main results and the role of chance: The ZP-free culture method was applied to two patients (patient A and B) with recurrent failure of ART in our clinic due to poor-quality embryos and/or difficulties in obtaining good quality blastocysts. In both cases, blastocysts were successfully obtained and cryopreserved for all ZP-free culture cycles. In patient A, one good quality ZP-free blastocyst was freshly transferred five days after oocyte retrieval, and a live male baby (2925g) was delivered at 40 weeks of gestation by caesarean section). In patient B, a frozen/thawed ZP-free blastocyst transfer was conducted, and a live female baby (3225g) was delivered at 39 weeks of gestation by vaginal delivery. This shows ZP-free culturing may help obtain viable embryos in patients for which conventional in vitro culturing methods result in embryos characterized with severe cytoplasmic fragmentation and poor quality in the early cleavage stage.
Limitations, reasons for caution: Although successful pregnancies and deliveries were confirmed in two cases, postnatal evaluations will be absolutely necessary for infants derived from ZP-free culture. In addition, the number of trial cases needs to be expanded, however careful selection of suitable patients is necessary for this novel culture method.
Wider implications of the findings: We found removing the ZP at the pronuclear stage improved embryo development and led to successful pregnancies and deliveries after blastocyst transfer. This indicates ZP-free culturing may be an effective method for decreasing cytoplasmic fragmentation caused by perivitelline threads or adhesion between the ooplasm and the zona pellucida.
Trial registration number: not applicable

P-217 The mitochondrial DNA copy number of cumulus granulosa cells is associated with the symmetry of cleavage embryo but not blastocyst quality

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Study question: To study the relationship between mitochondrial DNA copy number of cumulus granulosa cells (CGCs-mtDNA) and the quality of early embryos.
Summary answer: CGCs-mtDNA was related to the symmetry of cleavage. However, CGCs-mtDNA was not associated with fertilization, blastocysts quality, or blastocysts euploidy.
What is known already: The potential of early embryonic development mainly depends on the quality of oocytes to a large extent. Mitochondria of CGCs are directly involved in the establishment of oocytes capacitation during oocytes maturation and development.
Study design, size, duration: This is a retrospective study from December 2018 to January 2019, involving a total of 283 CGCs surrounding Metaphase II oocytes from 49 patients who underwent preimplantation genetic testing for aneuploidy (PGT-A) at the Reproductive and Genetic Hospital of CITIC-Xiangya.
Participants/materials, setting, methods: We used the TaqMan probes to quantitatively detect mitochondrial DNA copy number of per CGCs by quantitative PCR in mitochondrial genes (MT-ND1 and MT-CO1) and a nuclear gene (-globin). Besides, according to the nature of the dependent variable, the binary logistic regression model and the logistic regression analysis model of ordered multi-classification were used for multivariate statistical analysis.
Main results and the role of chance: The CGCs-mtDNA corresponding to fertilized eggs was not different from that of unfertilized eggs in MT-ND1 gene and MT-CO1 gene (MT-ND1: fertilized vs. unfertilized, 600±337 vs. 604±367, P=0.593; MT-CO1: fertilized vs. unfertilized, 1336±531 vs. 1329±478, P=0.938). Interestingly, we found that the CGCs-mtDNA of D3 embryos with good quality was statistically higher than that of D3 embryos with fair or poor quality for MT-ND1 gene and MT-CO1 gene (MT-ND1: good quality vs. fair/poor quality, 803±627 vs. 587±307, P=0.028; MT-CO1: good quality vs. fair/poor quality, 1682±554 vs. 1374±702, P=0.025). Moreover, the CGCs-mtDNA corresponding to D3 embryos with even cleavage was higher than that of D3 embryos with uneven cleavage (MT-ND1: even cleavage vs. uneven cleavage, 803±627 vs. 590±309, P=0.036; MT-CO1: even cleavage vs. uneven cleavage, 1562±552 vs. 1316±525, P=0.037). Besides, we investigated the difference among the CGCs-mtDNA in blastocysts with the good quality, blastocysts with fair or poor quality, and the developmental blocked embryos before the blastocyst stage. But we didn't found any difference among the above three groups (MT-ND1: P=0.531; MT-CO1: P=0.609). In the study of the relationship between CGCs-mtDNA and blastocysts euploidy, we got similar results (MT-ND1: P=0.602; MT-CO1: P=0.570).
Limitations, reasons for caution: The sample size of this study was relatively small.
Wider implications of the findings: Although the sample size of this study is limited, our results indicated the importance of mitochondria in CGCs in early embryo development, especially in the first three days. The investigation of mitochondrial function in CGCs may shed light on the mechanism of CGCs-oocyte crosstalk.
Trial registration number: LL-SC-2019-005

P-218 Analysis of the occurrence of microbial contamination in IVF culture system and the effect of microorganisms on embryo development and clinical outcomes

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Study question: what is the source, prevalence, and influence of microbial contamination on in vitro fertilization (IVF) and embryo transfer (ET) cycles?
Summary answer: Microbial contamination mainly occurs on Day 2, most caused by *Escherichia coli* carried with semen. ICSI could prevent contamination effectively and get good clinical outcomes.
What is known already: Microbial contamination occurs in IVF-ET system occasionally, which is hard to stop happening. The IVF culture system and laboratory environment, the patients' follicular fluid and semen are not absolutely sterile, while the antibiotics in culture medium isn't effective for all microbe types, and the artificial operations may bring in microbes. Generally, microbial contamination leads to degradation of embryos, reduction the number of embryos available, and infection of female reproductive tract, which would increase the cost of patients' time, money, and bring psychological damages. A better understanding of embryo contamination in IVF culture system is of added value.
Study design, size, duration: A total of 29583 IVF-ET cycles were enrolled in this prospective observational study, from January 2010 to December 2020, included 70 microbial contamination cycles discovered in Day1-Day3 (D1-D3) of in vitro culture. Follicular fluid and semen saved on oocyte retrieval day, and culture medium contaminated were examined and identified for microorganisms at each contamination cycle.
Participants/materials, setting, methods: Compared the contamination rate of different insemination methods (IVF/ICSI/IVF+ICSI), different in vitro culture days (D1-D3), and different samples examination (follicular fluid, semen, culture medium) respectively, identified the source of microorganism types, compared the IVF culture outcomes and clinical outcomes between total contamination group (TC group, 42 cases) and partial contamination group (PC group, 28 cases).
Main results and the role of chance: A total of 70 microbial contamination cases occurred in 29583 oocyte retrieving cycles (0.24%), and it was observed only in IVF embryos but never in ICSI (Intracytoplasmic sperm injection) embryos. 38 contamination cases occurred on D2 with a highest ratio (54.3%) compared to D1 (32.9%) and D3(12.9%); Compared with follicular fluid, semen was the main cause inducing contamination from D1 to D3, and *Escherichia coli* in semen and culture medium, *Enterococcus faecalis* in follicular fluid proved to be the most common sources. Compared with TC group, the PC group showed a lower rate of No-available embryos (21.4% vs 81.0%) and a higher rate of blastocyst formation (41.2% vs 28.6%), In addition, the clinical pregnancy rate of PC group was higher than that of TC group in both fresh and frozen-thawed embryo transfer cycles (31.3% vs 16.7%, 38.5% vs 0.0%).
Limitations, reasons for caution: Further study is still necessary to better understand the sources that induce microbial contamination embryos, and more efficient methods are required to remove the microbes on these contaminated embryos so as better develop and manage a sterile micro-environment for successful embryo growth.
Wider implications of the findings: The differential embryonic microbe types associated to different IVF culture and clinical outcomes in patients undergoing IVF-ET might have profound implications for understanding the microbial sources and making a better management of IVF culture system.
Trial registration number: Not applicable

P-219 mtDNA content in bovine cumulus cells does not predict oocyte´s developmental competence

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Study question: Does cumulus cell mtDNA content correlate with oocyte developmental potential in the bovine model?
Summary answer: The relative amount of mtDNA content did not vary significantly in oocytes showing different developmental outcomes following IVF
What is known already: Cumulus cells are closely connected to the oocyte through transzonal projections, serving essential metabolic functions during folliculogenesis. These oocyte-supporting cells are removed and discarded prior to ICSI, thereby constituting an interesting biological material on which to perform molecular analysis aimed to predict oocyte developmental competence. Previous studies have positively associated oocyte´s mtDNA content with developmental potential in both animal models and women. However, it remains debatable whether mtDNA content in cumulus cells could be used as a proxy to infer oocyte developmental potential.
Study design, size, duration: Bovine cumulus cells were allocated into three groups according to the developmental potential of the oocyte: 1) oocytes developing to blastocysts following IVF (Bl+Cl+), 2) oocytes cleaving following IVF but arresting their development prior to the blastocyst stage (Bl-Cl+), and 3) oocytes not cleaving following IVF (Bl-Cl-). Relative mtDNA content was analysed in 40 samples/group, each composed by the cumulus cells from one cumulus-oocyte complex (COC).
Participants/materials, setting, methods: Bovine cumulus-oocyte complexes were obtained from slaughtered cattle and individually matured *in vitro*

(IVM). Following IVM, cumulus cells were removed by hyaluronidase treatment, pelleted, snap frozen in liquid nitrogen and stored at -80 °C until analysis. Cumulus-free oocytes were fertilized and cultured *in vitro* individually and development was recorded for each oocyte. Relative mtDNA abundance was determined by qPCR, amplifying a mtDNA sequence (*COX1*) and a chromosomal sequence *(PPIA*). Statistical differences were tested by ANOVA.

Main results and the role of chance: Relative mtDNA abundance did not differ significantly (ANOVA p>0.05) between the three groups exhibiting different developmental potential (1±0.06 vs. 1.19±0.05 vs. 1.11±0.05, for Bl+Cl+ vs. Bl-Cl+ vs. Bl-Cl-, mean±s.e.m.).

Limitations, reasons for caution: Experiments were conducted in the bovine model. Although bovine folliculogenesis, monoovulatory ovulation and early embryo development exhibit considerable similarities with that of humans, caution should be taken when extrapolating these data to humans.

Wider implications of the findings: The use of molecular markers for oocyte developmental potential in cumulus cells could be used to enhance success rates following single-embryo transfer. Unfortunately, mtDNA in cumulus cells was not found to be a good proxy for oocyte quality.

Trial registration number: not applicable

P-220 Delayed-ICSI on day1-matured oocytes in low responders of different age groups

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Study question: Is the cycle outcome of day0-ICSI (day0-matured oocytes) and delayed-ICSI (day1-matured oocytes) in low responders affected by maternal age?

Summary answer: Delayed-ICSI improves cycle outcome of low responders in all age groups by providing additional blastocysts for embryo transfer and/or pre-implantation genetic testing for aneuploidies (PGT-A).

What is known already: We had previously compared the fertilization, blastulation, blastocyst utilisation, and euploidy rates between day0-ICSI and delayed-ICSI (Lee, C.S.S., 2018). We believed that patients who have <7 mature oocytes (low responders) can benefit by having their concomitant immature oocytes cultured to day 1. This study evaluates the benefit of delayed-ICSI in improving the cycle outcome of such low responders from different age groups.

Study design, size, duration: From January 2018 to December 2020, 434 IVF cycles in Alpha IVF & Women's Specialists were classified as low responder (being <7 oocytes injected on day 0). The immature oocytes were further cultured and delayed-ICSI was done on day1-matured oocytes. Patients were divided into 3 groups: (A) ≤35 years old (n=137; mean maternal age=32.5; range=23.0-35.0); (B) 36-40 years old (n=208; mean maternal age=38.2; range=36.0-40.0); and (C) >40 years old (n= 89; mean maternal age=42.7; range=41.0-49.0).

Participants/materials, setting, methods: Semen samples were processed by density gradient centrifugation and/or swim-up method. Day0-matured oocytes were injected at 2.5-4.5 hours post-retrieval (PIEZO, Japan). Immature oocytes were further cultured for 18-24hours and delayed-ICSI was done immediately after maturity assessment. Injected oocytes were cultured up to 7 days and trophectoderm biopsy for PGT-A screening (IonTorrent, USA) was performed on selected blastocysts prior to vitrification (Cryotec, Japan). The cycle outcomes of day0-ICSI and delayed-ICSI were analysed and compared.

Main results and the role of chance: In Group A, the fertilisation, blastulation, blastocyst utilisation and euploidy rates of day0-ICSI and day1-ICSI were 80.0% vs. 70.9%, 74.7% vs. 33.6%, 57.1% vs. 17.9%, and 52.3% vs. 30.8% respectively. The fertilisation (p=0.0024), blastulation (p=0.0001), utilization (p=0.001) and euploidy (p=0.0205) rates of delayed-ICSI were significantly lower compared to day0-ICSI.

In Group B, the fertilisation, blastulation, blastocyst utilisation and euploidy rates of day0-ICSI and day1-ICSI were 77.3% vs. 64.1%, 75.0% vs. 37.5%, 52.7% vs. 20.6% and 26.0% vs. 32.1% respectively. Delayed-ICSI showed significantly lower fertilisation (p=0.0001), blastulation (p=0.0001) and utilization (p=0.0001) rates than day0-ICSI, but the euploidy rate was comparable (p=03997).

In Group C, the fertilisation, blastulation and blastocyst utilisation rates of day0-ICSI and delayed-ICSI were 76.6% vs. 56.4%, 67.1% vs. 22.8%, 34.3% vs. 7.9% respectively. Similar to Group B, the fertilization (p=0.0001), blastulation (p=0.0001) and utilisation (p=0.0003) rates in day0-ICSI was significantly higher than delayed-ICSI. No significant difference was observed between the euploidy rates of day0-ICSI and delayed-ICSI (18.9% vs. 0.0%, p=0.3452). Despite all blastocysts derived from delayed-ICSI in this group being aneuploid, it could still increase the chances of these patients obtaining an euploid blastocyst.

Limitations, reasons for caution: Analysis on Group B was done on a larger sample size compared to Group A and C. A larger sample size in Group A and C is needed to further support our results.

Wider implications of the findings: Delayed-ICSI generates additional blastocysts for low responders from all age groups. Therefore, delayed-ICSI could be a routine procedure in low responder IVF patients in order to optimise the cycle and clinical outcomes.

Trial registration number: not applicable

P-221 Prospective randomized sibling study on gamete preparation, insemination and subsequent culture of human oocytes in a time-lapse system using media systems with and without antioxidants

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Study question: Does the addition of antioxidants for gamete preparation, insemination and embryo culture lead to differences in embryo development and clinical outcome

Summary answer: Using an antioxidant-containing media system for sperm preparation, insemination and embryo culture imparts significantly higher good-quality blastocyst rates and improved clinical outcome in elderly patients.

What is known already: A previous study showed that adding combined antioxidants for sequential embryo culture in conventional incubators (interrupted culture) improves embryo viability and clinical outcome, especially for elderly patients. Here we investigated the combined effect of three antioxidants Acetyl-L-Carnitine (10 µM), N-Acetyl-L-Cysteine (10 µM), and α-Lipoic Acid (5 µM) during sperm preparation, insemination, and time-lapse culture in a single step medium on human embryo development and clinical outcome.

Study design, size, duration: Prospective randomized single center study including 143 couples for IVF/ICSI between August 2018 and December 2019. Inclusion required at least eight cumulus-oocyte-complexes (COCs) after retrieval. Cycles involving PGT, split IVF/ICSI, and surgically retrieved sperm were excluded. Immediately after retrieval oocytes were randomly distributed to a study or control media system with or without antioxidants (Vitrolife). Similarly, ejaculates were split and prepared with and without antioxidants.

Participants/materials, setting, methods: Sibling oocytes were inseminated in the respective group with accordingly prepared sperm. Single step embryo culture was conducted in medium with (Gx-TL) and without (G-TL) antioxidants in the EmbryoScope+. Embryo quality and clinical outcome were assessed in relation to maternal age (<35/>35 years). Good-quality embryos on day 3 were defined as 8- to 10-cells with even cells and low fragmentation; good-quality blastocysts as >3BB. Clinical outcome was assessed after single vitrified blastocyst transfer (SVBT).

Main results and the role of chance: From 143 participants (female age, 34.7±3.2 years), a total of 2424 COCs were collected; 1180 COCs/916 metaphase-II (MII) oocytes were allocated to Gx-TL media and 1244 COCs/981 MII oocytes to G-TL media. Age-related analysis in Gx-TL compared with G-TL in relation to allocated MII oocytes revealed a trend for higher fertilization rates in Gx-TL for both age groups (<35: 72.1% vs. 66.9%; >35: 70.7% vs. 64.9%, P<0.1). Good-quality day 3 embryo development/MII oocytes was higher, albeit not significant, in the elderly patients in Gx-TL (<35: 35.9% vs. 34.4%; >35: 31.1% vs. 27.9%). Overall day 5/6 blastocyst rate was similar for both media (<35: 48.2% vs. 49.9%; >35: 42.3% vs. 39.5%). Day 5/6 GQB rate was comparable for younger patients (<35: 23.8% for Gx-TL vs. 26.0% for G-TL) but significantly higher in Gx-TL in elderly patients (>35: 20.7% vs. 14.4%; P<0.05). A total of 200 SVBT were performed; 99 in the Gx-TL- and 101 in the G-TL-arm. We

noted almost similar implantation and ongoing pregnancy rates between Gx-TL vs G-TL in the younger (<35) age group (50.0% vs. 55.4%; 50.0% vs. 55.6%) but higher albeit not significant rates for Gx-TL in older (>35) patients (44.1% vs. 33.3%; 44.1% vs. 33.3%).
Limitations, reasons for caution: In almost 95% of the cycles, oocytes were inseminated by ICSI; thus results may not equally apply for cycles with IVF. The use of a closed time-lapse system may have prevented from some environmental oxidative stress. Therefore results may come out different with a similar study using standard incubation.
Wider implications of the findings: Supplementation of antioxidants to media for gamete isolation and preparation, as well as subsequent single step time-lapse culture may improve GQE/B rates and clinical outcomes in certain age groups, plausibly through the reduction of oxidative stress. Further studies in selected sub-groups (severe OAT syndrome / testicular cases) may be indicated.
Trial registration number: UMIN000034482

P-222 Can we optimise the time that we perform the fertilisation check in the lab? Lessons learnt from time-lapse incubation

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Study question: Can time-lapse data be used to identify the optimum time to perform the fertilisation check for oocytes cultured in standard incubation?
Summary answer: The optimum time to perform fertilisation checks for oocytes cultured in standard incubation is 16.5hpi+/-0.5h.
What is known already: Time-lapse incubation allows the embryologist to retrospectively review collated images of oocytes and embryos to capture important embryological observations that may have otherwise been missed. This is a luxury not available to embryologists when oocytes or embryos are cultured in standard incubation. Traditionally, the optimum time to perform the fertilisation check is 17 hours post insemination (hpi) +/- 1 hour. It was hypothesised that this could be fine-tuned ensuring the maximum number of fertilised oocytes were observed, thereby increasing the number of usable embryos for the patient.
Study design, size, duration: This was a retrospective, multicentre analysis including data from 27,022 ICSI derived embryos cultured in time-lapse incubation between January 2011 to November 2019.
Participants/materials, setting, methods: The time of pronuclei appearance and disappearance was recorded using the time-lapse incubation software. The number of oocytes exhibiting normal fertilisation (defined as the presence of two pronuclei) during 30 minute intervals from 15hpi to 20hpi was determined.
Main results and the role of chance: Between 15-17.5hpi the average number of oocytes exhibiting normal fertilisation was 98.19% with most oocytes having visible pronuclei at 16-16.5hpi (98.32%). At 18-18.5hpi the number of visible pronuclei falls to 95.53% and continues to fall to 87.02% at 19.5-20hpi meaning that over 3000 (11%) normally fertilised oocytes, within this cohort, would not be identified.
Limitations, reasons for caution: The conclusions of this investigation cannot be effectively extrapolated to IVF embryos as only ICSI embryos were used for the determination of the results.
Wider implications of the findings: The optimum time to perform fertilisation checks for oocytes cultured in standard incubation is 16.5hpi+/-0.5h. However, without the use of time-lapse incubation, the fertilisation of at least 2% of embryos that create a fetal heart will be missed, even if the fertilisation check is performed in the optimum window (16.5hpi+/-0.5h).
Trial registration number: Not applicable

P-223 The necrotic oocyte: does the uncontrolled release of cell contents affect adjacently, group cultured embryos?

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Study question: Is embryo utilisation rate, embryo morphokinetics and the incidence of irregular divisions affected when embryos are group-cultured adjacent to a necrotic oocyte?
Summary answer: This study demonstrates that embryos cultured adjacent to necrotic oocytes appear to be unaffected both in terms of utilisation, morphokinetics and incidence of irregular divisions.
What is known already: Necrosis is a form of uncontrolled cell death, usually resulting from external injury, causing the cell's contents to release into the surrounding environment1. A cell undergoing necrosis will first visibly swell before the collapse of the plasma membrane causes it to subsequently shrink and the cell to lyse2. An escalation of inflammation occurs due to the release of intracellular factors3. Neighbouring embryos are believed to be negatively affected by a necrotic oocyte with some laboratories choosing to remove necrotic oocytes from culture dishes, however, little is known regarding this impact.
Study design, size, duration: The project was a single site, retrospective cohort analysis using time-lapse data from August 2017 to December 2018. Only patients with at least one necrotic oocyte, a minimum of one adjacent embryo to the necrotic oocyte and those cultured in the EmbryoScope+® were included in the analysis.
Participants/materials, setting, methods: The study included 868 embryos from 89 patients. The embryos were categorised as adjacent to a necrotic oocyte (group 1, n=208) and not adjacent to a necrotic oocyte (group 2, n=660). The utilisation rate and irregular division rate were analysed using a Chi-squared test, the morphokinetic parameters was analysed using a t-test. Morphokinetic data included; tPB2, tPNa, tPNf, t2, t3, t4, t5, t6, t7, t8, t9, tSC, tM, tSB and tB.
Main results and the role of chance: Utilisation rate between the two groups was not significantly different (group 1; 40.9% versus group 2; 47.6%, p=0.09). Incidence of irregular division was not significantly different between the two groups (group 1; 24.0% vs group 2; 21.7%, p= 0.51). No morphokinetic parameter was statistically significantly different when comparing group 1 to group 2, respectively: tPB2, 3.61 vs 3.73, p= 0.38; tPNa, 7.01 vs 6.91, p= 0.59; tPNf, 23.64 vs 23.66, p= 0.95; t2, 3.44 vs 2.98, p= 0.09; t3, 14.56 vs 14.41, p= 0.75; t4, 15.96 vs 15.8, p= 0.77; t5, 15.96 vs 15.8, p= 0.77; t6, 30.33 vs 30.46, p= 0.86; t7, 33.11 vs 33.16, p= 0.95; t8, 37.93 vs 36.92, p= 0.34; t9, 48.66 vs 48.97, p= 0.73; tSC, 58.04 vs 57.89, p= 0.88; tM, 74.02 vs 73.76, p= 0.8; tSB, 75.55 vs 75.42, p= 0.9; tB, 87.06 vs 87.2, p= 0.91.
Limitations, reasons for caution: The time at which the oocytes became necrotic was not analysed therefore the effect, if any, of exposure time could not be determined. Of the 169 necrotic oocytes, two were from IVF and 167 from ICSI; the increased exposure of the embryos derived from ICSI was not controlled for.
Wider implications of the findings: Necrotic oocytes are easily identified in standard culture observations and in time-lapse imaging, therefore, their removal may be an unnecessary practice. More harm could be caused by removing the dish from the incubator, as this would unnecessarily expose any viable embryos contained within the dish to a suboptimal environment.
Trial registration number: Not applicable

P-224 Multinucleated and non-multinucleated embryos in PGT-A cycles: Is there any difference?

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Study question: Is multinucleation during cleavage stage correlated with the ploidy status of embryos and how does it affect the clinical outcome?
Summary answer: The presence of multinucleated embryos does not affect clinical outcome, although the risk of aneuploidy is higher in multinucleated embryos.
What is known already: Multinucleated blastomeres (MN) of cleavage stage embryos has been reported widely in scientific literature. Multinucleation has

been associated with diminished embryo developmental competency and clinical outcomes such as lower implantation. Although this is an intriguing subject of research, it is not clear yet whether multinucleation is related to aneuploidy. Morphological irregularities such as multinucleation in blastomeres became a constant finding only after the perpetually evolving technology of time-lapse culture of embryos which in combination with PGT-A analysis, creates new research paths which aim to develop a new tool for selection or deselection of embryos for transfer.

Study design, size, duration: This retrospective study, included 97 PGT-A cycles, performed at Embryolab fertility clinic from May 2017 to December 2020, all cultured in time-lapse incubator (EmbryoScope). Two study groups were formed; the MN Group consisted of PGT-A cycles with at least one multinucleated embryo (n=56) and the Control Group in which all PGT-A cycles had no multinucleated embryos (n=38). Euploidy rate, type of chromosomal abnormality, cumulative pregnancy and live birth rates were compared between the two groups.

Participants/materials, setting, methods: Embryos were annotated for the existence of multinucleated blastomeres on Day 2 of their development. Biopsy was performed on Days 5/6 and embryos were genetically tested. One or two euploid embryos were transferred. Euploidy rate and clinical outcomes between the two groups were compared. Within the MN group, euploidy rate between multinucleated and non-multinucleated embryos was compared. For abnormal embryos, association of multinucleation with the type of abnormality was tested. SAS statistical analysis was performed.

Main results and the role of chance: Mean female age was 35.93 years in the MN group and 38.39 years in the control group. Blastocyst formation rate (expressed per fertilised oocytes) was similar between MN and Control group (74% vs 76%, p=0.6303). In the MN group, 56 cases resulted in 44 embryo transfers while in the control group 38 cases resulted in 23 embryo transfers. Pregnancy rates (59.09% vs 65.21%, p=0.6255) and clinical pregnancy rates (45.45% vs 39.13%, p=0.4245) were not significantly different between MN and Control group. Initially, cumulative live birth rate was found to be significantly higher in the MN group compared to the Control group (62.96% vs 33.34%, p=0.0417). However, when logistic and poisson regression was applied, it became obvious that this difference was not affected by multinucleation but from other factors such as female age. When comparing multinucleated and non-multinucleated embryos within the MN group, it was found that the mean number of euploid embryos was significantly higher in the non-multinucleated subgroup of embryos (p=0.0021). No correlation was found between multinucleation and the type of chromosomal abnormality.

Limitations, reasons for caution: The sample size is the main limitation of the present study. More research with bigger sample size is needed in order to confirm the finding of the present study.

Wider implications of the findings: The present study suggests that multinucleated blastomeres during embryo development is not an indication for diminished blastocyst formation and does not affect the clinical outcomes. However, within a sibling embryo population, non-multinucleated embryos tend to be euploid and this finding can be used to advance embryo selection efficiency.

Trial registration number: not applicable

P-225 The effect of rapid and delayed insemination on reproductive outcome in conventional insemination and intracytoplasmic sperm injection invitro-fertilization cycles.

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Study question: Does rapid or delayed insemination after egg retrieval affect fertilization, blastocyst development and live birth rates in CI and ICSI cycles?

Summary answer: When performing CI or ICSI <1.5h and >6.5h after retrieval, detrimental effects are moderate on fertilization but do not impact blastocyst usage and birth rates.

What is known already: Several studies have shown that CIor ICSI performed between 3 to 5 h after oocyte retrieval has improved laboratory outcomes. However, some studies indicate that insemination of oocytes, by either CI or ICSI, within 2 hours or more than 8 hours after oocyte retrieval has a detrimental effect on the reproductive outcome. With some ART centres experiencing an increase in workload, respecting these exact time intervals is frequently challenging.

Study design, size, duration: A single-center retrospective cohort analysis was performed on 6559 patients (9575 retrievals and insemination cycles) between January 1st2017 to July 31st2019. The main outcome measures were live-birth rates. Secondary outcomes included analysis of fertilization per all oocytes retrieved, blastocyst utilization, clinical pregnancy, and miscarriage rates. All analyses used time of insemination categorized in both CI and ICSI cycles. Fertilization rates across categories was analyzed by ANOVA and pregnancy outcomes compared using Chi-square tests.

Participants/materials, setting, methods: As part of laboratory protocol, oocyte retrieval was performed 36 h post-trigger. Cycles involving injection with testicular/epididymal sperm, donor or frozen oocytes were excluded. The time interval between oocyte retrieval and insemination was analyzed in eight categories: 0 (0- <0.5h), 1 (0.5-<1.5h), 2 (1.5-<2.5h), 3 (2.5-<3.5h), 4 (3.5-<4.5), 5 (4.5-<5.5), 6 (5.5-<6.5) and 7 (6.5-<8h). The number of retrievals in each group (0-7) was 586, 1594, 1644, 1796, 1836, 1351, 641 and 127 respectively.

Main results and the role of chance: This study had a mean patient age of 36.0 years and mean of 12.2 oocytes per retrieval in each category. There were 4,955 CI and 4,620 ICSI retrievals. The smallest groups were time category 7 and 0 for CI and ICSI respectively. The results showed that the mean fertilization rate per egg retrieved for CI ranged from 54.1 to 64.9% with a significant difference between time category 0 and 5 (p<0.001) and category 1 and 5 (p<0.0.001). Mean fertilization rate for ICSI per egg retrieved ranged from 52.8 to 67.3% with no significant difference between time categories compared to category 5. Blastocyst utilization rate for CI and ICSI were not significantly different for all time categories. In the CI and ICSI groups there were 6,540 and 6,178 total fresh and frozen transfers. The miscarriage and clinical pregnancy rate in CI and ICSI were not significantly different across time categories. The overall mean live birth rate for CI was 32.4% (range: 23.1 to 35.5%). Live-birth rates differed significantly (p=0.04) in CI with time categories 0 and 7 the lowest. In the ICSI group, the overall mean live birth rate was 30.8% (range: 29.1 to 35.7%),with no significant differences between time categories.

Limitations, reasons for caution: As this is a retrospective study, the influence of uncontrolled variables cannot be excluded. The group spread was uneven with the early and late time categories having the lowest number of representative retrievals and this could have affected the results obtained.

Wider implications of the findings: Our results indicate that both CI and ICSI are optimal when performed between 1.5-6.5 hours after oocyte retrieval. Further prospective studies on reproductive outcomes related to time of insemination are warranted. This data indicates a minimal detrimental effect when it is untenable to follow strict insemination time intervals.

Trial registration number: 2015P000122

P-226 Failure of blastocoele expansion within the first two hours post thawing could halve the chances of implantation

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Study question: The aim of this study was to evaluate the influence of blastocoele re-expansion time of warmed vitrified blastocysts on clinical pregnancy outcome.

Summary answer: Clinical pregnancy rate was significantly higher after transfer of warmed vitrified blastocysts that were fully expanded within 2 hours post thaw.

What is known already: The number of blastocysts being vitrified worldwide has increased dramatically over recent years. A combination of factors has led to this including the introduction of vitrification, an increase in freeze-all policies, single embryo transfer and an increase in preimplantation genetic testing. Currently, blastocyst re-expansion after thawing is used to indicate the survival status of the blastocyst and when combined with the morphology of blastocyst can predict its reproductive potential. While time taken for blastocoele re-expansion has been proposed to be a biomarker of viability, its value in clinical practice remains unclear.

Study design, size, duration: This retrospective study analysed outcomes in patients who had frozen embryo transfers between June-December 2020. 233 embryos were reviewed with time-lapse to assess their blastocoele expansion post-warming and three groups were identified. The first included fully expanded blastocysts post-warming. The second group included partially expanded blastocysts and the third non-expanded blastocysts. In addition, the groups were subcategorised into two further categories depending on whether they took less or more than 2 hours to complete expansion.
Participants/materials, setting, methods: 233 vitrified/warmed embryos from 216 patients were analysed using time-lapse incubators. The first group included 134 blastocysts, of which 70 were fully expanded within 2 hours and 64 after 2 hours post thaw. The second group had 70 embryos of which 45 expanded partially within 2 hours and 25 after 2 hours. The third had 28 embryos that had no expansion within the first 2 hours (n=20) or after 2 hours (n=8).
Main results and the role of chance: Blastocysts were collapsed by laser prior to vitrification. Single blastocyst transfer was performed for all patients. The mean transferred embryo age was 32.1± 5.5 and the recipient's was 37.5± 5.9. Fully expanded blastocysts (n=70) within 2 hours demonstrated a clinical pregnancy rate (CPR) of 57% compared with 38% from those that expanded fully after 2 hours (n=64) (p=0.02). Blastocysts with some form of expansion (full or partial) within 2 hours post-warming (n=115) were associated a significantly higher CPR compared to those expanding after 2 hours (n=89). The CPR was 55% and 39% respectively (p=0.02). Embryos that showed no expansion (n=20) within the first 2 hours post thaw resulted in CPR of 28%. Interestingly, embryos that showed no expansion after 2 hours resulted in no pregnancy. When combining morphology as a selection criterion, expansion within 2 hours of thawing was associated with a CPR of 62.5% for ≥4AB embryos, 50% for BB embryos and 45% for poorer embryos ≤CB.In conclusion, failure of blastocoele expansion post 2 hours reduced by half the chances of clinical pregnancy (p=0.03). Combination of the degree of re-expansion and embryo morphology is an important predictor tool to improve clinical outcomes in frozen embryo transfers.
Limitations, reasons for caution: This study uses a small sample size of patients. The data are observational and were retrospectively analysed so unknown confounders could not be assessed. The addition of more cycles and further multivariate analysis, is essential for confirmation of the findings. However, initial results are very reassuring.
Wider implications of the findings: The degree of speed of re-expansion post warming should be used as a predictor for prioritisation of embryos for transfer. Owing to these preliminary findings there is rationale for a larger scale study combining other morphological indicators that could further assess implantation indicators and assist patient counselling
Trial registration number: Not Applicable

P-227 Fatty acid regulation of Nrf2/Keap1 pathway during mouse preimplantation embryo development

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Study question: Our objective is determining whether supplementing embryo culture media with palmitic acid and/or oleic acid impacts Nrf2/Keap1 antioxidant response pathways during preimplantation mouse embryo development.
Summary answer: Supplementation of embryo culture media with palmitic acid increases cellular Nrf2 levels per embryo after 48-hour culture, while oleic acid reverses this effect.
What is known already: Obese women experience higher incidence of infertility than women with healthy BMIs. The obese reproductive tract environment supporting preimplantation embryo development is likely to include enhanced free fatty acid (FFA) levels and increased accumulation of reactive oxygen species. Exposure to palmitic acid (PA) *in vitro* significantly impairs mouse embryo development while increasing ER stress mRNAs. Oleic acid (OA) reverses these effects. To further define effects of FFA exposure, we are characterizing the influence of FFAs on the Nrf2–Keap1 pathway and its downstream antioxidant defense systems. We hypothesize that PA treatment induces Nrf2-Keap1 activity, while OA treatment alleviates pathway activity.
Study design, size, duration: Female CD-1 mice (4–6 weeks) were super-ovulated via intraperitoneal injections of PMSG, followed 48 hours later by hCG. Female mice were mated with male CD-1 mice (6–8 months) overnight. Females were euthanized using CO2 and two-cell embryos were collected by flushing oviducts. Two-cell embryos were placed into KSOMaa-based treatment groups: 1) BSA (control); 2) 100μM PA; 3) 100μM OA; 4) 100μM PA+OA, and cultured for 48 hours (37°C; 5% O2, 5% CO2, 90% N2).
Participants/materials, setting, methods: After 48-hour embryo culture, developmental stages of all mouse embryos were recorded. Immunofluorescence analysis of Nrf2 and Keap1 localization was performed for embryo treatments (BSA, 100μM PA, 100μM OA & 100μM PA+OA) using rabbit polyclonal anti-Nrf2 antibody, with Rhodamine-Phalloidin and DAPI staining. Embryos were imaged using confocal microscopy and Nrf2-positive cells were counted using ImageJ. Nrf2 and Keap1 mRNA abundances were assessed after culture in each treatment condition using RT-qPCR and the delta-delta Ct method.
Main results and the role of chance: Inclusion of 100μM PA in embryo culture significantly decreased blastocyst development frequency from 70.06±16.38% in the BSA (control) group to 11.61±8.19% in the PA-treated group (p<0.0001). Embryo culture with 100μM OA and 100μM PA+OA co-treatment did not significantly impair blastocyst development (OA: 61.59±8.07%, p=0.4053; PA+OA: 63.53±7.63%, p=0.6204).

Embryo culture with PA treatment significantly increased the mean percentage of Nrf2-positive cells to 56.83±30.49% compared with 21.22±15.63% in the control group (p<0.0001). Conversely, 100μM OA and 100μM PA+OA treatments did not significantly affect Nrf2-positive cell frequencies compared with the control group (OA: 33.28±21.83%, p=0.1825; PA+OA: 34.84±12.66%, p=0.0691). Immunofluorescence results show that treating embryos with 100μM PA for 48 hours results in increased levels of cellular Nrf2, while combining 100μM PA with 100μM OA reversed these effects.

Preliminary qPCR analysis showed no significant differences in Nrf2 or Keap1 relative transcript abundance between any embryo treatment groups. Nrf2 and Keap1 mRNA levels were both higher after embryo culture with 100μM OA than all other culture groups (p=0.6268; p=0.3201). Notably, Keap1 relative transcript levels dropped to undetectable levels after culture with 100μM PA, which suggests an increase in Nrf2 activation.Limitations, reasons for caution: While immunofluorescence localization of Nrf2/Keap1 provides insight into how the proteins behave during preimplantation embryo development, confocal images cannot determine protein-protein interactions or activity levels. Similarly, transcript information from RT-qPCR analysis only provides information about Nrf2 and Keap1 at the transcript level. Nrf2 activity will be assessed via downstream targets.
Wider implications of the findings: The Nrf2–Keap1 pathway coordinates numerous cellular defence mechanisms, and is implicated in various diseases, including cancer. Establishing an impact of free fatty acid exposure on Nrf2–Keap1 during preimplantation embryo development will provide valuable information regarding the effects of maternal obesity on outcomes for embryos produced from these patients.
Trial registration number: Not applicable

P-228 AI-based assessment of embryo viability correlates with features of embryo ploidy

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Study question: Do AI models used to assess embryo viability (based on pregnancy outcome) also correlate with known embryo quality measures such as ploidy status?
Summary answer: An AI for embryo viability assessment correlated with ploidy status, and with karyotypic features of aneuploidy, supporting its use for embryo selection.
What is known already: One factor that can influence pregnancy success is the genetic status of the embryo. PGT-A is commonly used to test for embryo ploidy, with the aim of identifying karyotypically normal embryos (euploid

embryos), for preferential transfer. There is evidence suggesting that transfer of euploid embryos produces favorable clinical outcomes over aneuploid embryos.

Given the AI model was trained to evaluate clinical pregnancy, it was hypothesized that the score might also correlate with ploidy status, and with different types of aneuploidies. Little is known about morphological correlations with embryo ploidy status, so we also sought to explore this relationship.

Study design, size, duration: This study involved analysis of a retrospective dataset of single static Day 5 embryo (blastocyst) images with associated PGT-A results and AI viability scores. The dataset comprised images of 5,469 embryos from 2,615 consecutive patients treated at five US IVF clinics between February 2015 and April 2020. The AI was trained on thousands of Day 5 embryo images from multiple IVF laboratories in multiple countries, but was not trained on data used in this study.

Participants/materials, setting, methods: Average patient age was 36.2 years, and average embryo cohort size was 2.1/patient. PGT-A analysis was performed on embryos at time of evaluation. The dataset comprised 3,251 (59.4%) euploid embryos, 1,815 (33.2%) aneuploid embryos, and 403 (7.4%) mosaic embryos. The AI was retrospectively used to provide a score between 0 (predicted non-viable) and 10 (predicted viable) for each image. Correlation between the AI viability score and euploid, mosaic and aneuploid embryos was then assessed.

Main results and the role of chance: Results showed a statistically significant correlation between AI viability score and PGT-A outcome, consistent with a relationship between pregnancy outcome and ploidy status. The average score for euploid embryos was 8.20, which was significantly higher than the average score for aneuploid embryos of 7.80 ($p<0.0001$).

There was a significant linear increase in confidence score from full aneuploid embryos, through mosaic embryos (average score 7.97), to full euploid embryos (mosaic threshold of 20-80%). High mosaic embryos tended to have a lower average score (7.60) than low mosaic embryos (7.96), consistent with correlation of viability (pregnancy outcome) with the degree of mosaicism. AI viability score also correlated with ploidy features believed to affect pregnancy outcomes. Trisomic changes had higher average scores than monosomic changes. Segmental changes had higher average scores than full gain or loss. The AI score differentiated euploid from aneuploid status more efficiently in embryos with poorer morphology than those with good morphology.

Whilst there was an evident correlation between pregnancy outcome and ploidy status, the AI was only weakly predictive of euploidy, with an accuracy of 57.3% using an AI viability score threshold of 7.5/10.This suggests pregnancy-related morphological features are somewhat correlated with embryo ploidy, but not completely.

Limitations, reasons for caution: The PGT-A technique is held to have some limitations for evaluating ploidy status, therefore it would be of benefit to perform additional confirmatory studies on independent datasets. It would be of interest to conduct prospective studies evaluating correlations between the AI's evaluation of morphology and pregnancy outcome with ploidy status.

Wider implications of the findings: The AI score correlated with genetic features of embryos that are known to correlate with pregnancy, which further supports the efficacy and use of AI for embryo viability assessment. The AI identified morphological features that are somewhat predictive of ploidy status, with potential application to embryos of poorer Gardner score.

Trial registration number: none

P-229 Oxidative stress and Metformin; an in-vitro study on serum and primary human granulosa cell cultures

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Study question: What is the effect of administration of Metformin on the oxidative stress (OS) levels in serum and primary human granulosa cell cultures of infertile females?

Summary answer: Metformin suppresses oxidative stress in serum and human granulosa cells and increases the expression of SIRT1 in OS induced environment.

What is known already: Oxidative stress (OS) is a resultant of mitochondrial dysfunction when it either fails to fight against the oxidants or the expression of the antioxidants is not sufficient. Cellular damage including DNA damage is a common resultant of oxidative stress. OS effects the oocyte maturation and moreover, the cleavage phase in the early embryonic stage. The raised levels of OS makers are hypothesized to compromise the nuclear maturation and the mitotic spindles of the maturing oocytes. Metformin seemed to decrease oxidative stress and improve insulin resistance, dyslipidaemia and endothelial dysfunction in PCOS patients

Study design, size, duration: This cross-sectional study was conducted from August 2017 – July 2019, at Aga Khan Hospital in collaboration with Australian Concept Infertility Medical Centre (ACIMC) on ten infertile patients undergoing egg retrieval after ethical approval from of Aga Khan Hospital (AKU-ERC-2018-0557-601).

Participants/materials, setting, methods: Serum samples were obtained and analysed. Follicular fluid of these subjects was collected for establishment of primary cell culture model of normal human granulosa cells (hGCs). Serum and hGC cultures were grouped as; a) control: treatment, b) Test1: H_2O_2 induced OS, and c) Test2: H_2O_2 induced OS treated with metformin. OS was estimated in all groups by Mishra method. The two Test groups were assessed for SIRT1 levels using quantitative PCR employing SIRT1 specific primers

Main results and the role of chance: With mean age of 32.04 ± 2.29 years the mean BMI was 27.61 ± 2.15 kg/m2. OS was induced and measured by an increase in optical density (OD) in hGC Test samples which showed 0.28 (0.16-0.40) OD when compared with control hGC samples 0.153 (0.09-0.23). There was a significant reduction in ODs after metformin treatment in the stress induced cells 0.182 (0.05-0.30). A similar pattern was observed in the serum samples in ODs; control: 0.105 (0.09-0.15), stress induced samples: 0.199 (0.19-0.20). and stress induced serum sample with metformin treatment: 0.1415 (0.06-0.18).

The Ct values obtained to express the effect of metformin on SIRT1 levels, for OS induced (Test1) and OS induced metformin treated (Test2) cells were found to be 29.12 and 26.42, respectively. We also observed a significant (85%) difference in the fold change of SIRT1 expression between metformin treated and untreated cells.

Limitations, reasons for caution: Small sample size is the limitation of this study. The impact of metformin on cell cultures due to different causes of infertility could not be ascertained

Wider implications of the findings: Metformin suppresses oxidative stress in serum and human granulosa cells and increases the expression of SIRT1 in OS induced environment, therefore, metformin may be considered as a treatment of oxidative stress in infertile patients. Randomized control trial with large sample size is recommended to confirm the cause and effect relationship.

Trial registration number: Not applicable

P-230 The NAD+ precursor nicotinamide riboside protects against postovulatary aging in vitro

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Study question: Can nicotinamide riboside, one of the NAD+ precursor, protect against postovulatary aging *in vitro?*

Summary answer: The NAD+ precursor nicotinamide riboside can protect against postovulatary aging *in vitro.*

What is known already: Postovulatory aging(POA) has been considered one of the most intractable challenges that limit the successful rate of ART. Multiple cellular and molecular changes have been involved during the process of POA. The NAD+ is a prominent redox cofactor which is indispensable to DNA repair, energy metabolism, autophagy, genomic stability as well as epigenetic homeostasis. Over the last several decades, increasingly studies have eported that NAD+ contents decline with age across multiple tissues and loss of it are implicated in various diseases associated to aging. As one of a precursor of NAD+, nicotinamide riboside play important role in regulating oxidative stress.

Study design, size, duration: In this study, we take advantage of *in vitro* aging model to explore the influences of NR administration on the postovulatory aged oocytes in mice. We analyzed the association of NR supplementation with the aging-related deterioration of oocyte quality, such as mitochondrial dysfunction, mislocalization of cortical granules, followed by embryonic development potential and the NAD+/SIRT1 signaling. We used 3582 oocytes totally.

Participants/materials, setting, methods: CD-1 mice oocytes/ *in vitro* culture/ UPLC-MS/MS for NAD+ contents measurement ,DCFH-DA staining for ROS detecting, γH2AX staining for DNA damage measurement, BODIPY FL ATP staining for ATP detecting, LCA-FITC staining to assess the distribution and dynamics of cortical granules (CGs), *In vitro* fertilization, Quantitative real time PCR

Main results and the role of chance: NR supplementation exerted protective effects on morphological defects of oocytes, and that these protective effects were concentration dependent. We detected a significantly decline in NAD+ levels in aging oocytes, however, NAD+ accumulation was present in aging oocytes after 200μM NR treatment, indicating that NR administration might be a feasible strategy to enhance the quality of aging oocytes. Furthermore, NR indeed elevated the embryonic development potential of POA oocytes after fertilization. Our findings revealed that NR administration effectively ameliorated ROS accumulation in POA oocytes.Then we tried to uncover the effects of NR on the meiotic apparatus in aging oocytes. NR supplementation can partially restores normal spindle assembly and chromosome alignment in postovulatory aging oocytes. Furthermore, we investigated the protective effects of NR on mitochondrial function through following expects: mitochondrial distribution, ATP production and mitochondrial membrane potential. NR treatment could promote mitochondrial function in oocytes during postovulatory aging *in vitro*. In addition, DNA damage and mislocalized CGs during postovulatory aging might be rescued by the supplementation of NR. Based on these evidences, we identified that NR improved the quality of aging oocytes by NAD+/SIRT1 axis.

Limitations, reasons for caution: Only in vitro, shown only in mice.

Wider implications of the findings: Our work represents a clinically effective pharmacological chemical to improve infertility caused by POA process. Further studies are needed to define the related mechanisms of NR supplementation on oocyte quality as well as reproductive outcomes clinically.

Trial registration number: N

P-231 Transnational oocyte donation program between Italy and Spain based on transport of vitrified oocytes: a five-years experience

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Study question: What is the clinical efficacy of an oocyte donation program based on the transportation of vitrified oocytes between two countries?

Summary answer: The transnational oocyte donation program is efficient, safe and comparable to other strategies (transport of frozen sperm and embryos).

What is known already: Egg donation represents a valid treatment strategy for women who have exhausted their ovarian function and it has considerably increased in the last years.

In Italy, egg donation is allowed after the judgment of the Constitutional Court n. 162 in 2014 but no reimbursement for the donors is provided. For this reason, the number of voluntary donors is irrelevant. Therefore, the great majority of egg donation cycles is carried out by using imported cryopreserved oocytes from foreign countries. However, recent evidence has questioned the overall efficacy of this strategy in comparison with the shipment of frozen sperm and vitrified embryos.

Study design, size, duration: A retrospective cohort study was conducted between July 2015-December 2020 at two private IVF clinics. 264 couples were treated (mean maternal age: 43.1± 4.6 years, range: 26–51; mean donor age: 24 ±3 years, range: 20-33) with vitrified oocytes shipped from a single Spanish egg bank (IMER, Valencia) to the receiving reproductive clinic in Italy (ANDROS Clinic, Palermo). All the oocytes for each batch were thawed.

Participants/materials, setting, methods: The primary outcome of this study was the cumulative clinical pregnancy rate (CPR) among the completed cycles for each batch of oocytes. Those cycles in which a clinical pregnancy was obtained, or all embryos derived by a single batch of oocytes had been transferred or no embryo was produced were defined as completed. In addition to main analyses, sensitivity analysis was performed to examine how the number of inseminated oocytes may affect CPR.

Main results and the role of chance: 2,367 oocytes in 355 batches were sent from Spain to Italy. 2,209 oocytes in 334 batches for 264 patients were thawed with a survival rate of 82.4% (1,821/2,209).

The mean number of oocytes received per patient was 6.6 ± 1.0. The fertilization rate was 72.1% (1,312/1,821). 499 embryos were transferred (38.0%), 335 at the cleavage stage (67.1%) and 164 at the blastocyst stage (32.9%); 197 supernumerary embryos were vitrified (15.0%), 18 at the cleavage stage (9.1%) and 179 at the blastocyst stage (90.9%). 616 embryos were not viable (47.0%). No more than two embryos were transferred for each embryo transfer (ET).

The completed cycles were 307 out of 334 (91.9%). The CPR per completed cycles was 46.6% (143/307) and 54.2% per patient (143/264). Clinical pregnancy rate per fresh ET in completed cycles with supernumerary cryopreserved embryos was significantly higher compared with that of the completed cycles without surplus embryos (56/101 versus 68/193, p=0.001). Logistic regression revealed that the number of inseminated oocytes was positively associated with CPR in a significant manner (B=0.220, p=0.007; OR=1.25, 95%CI=1.06-1.47). The multiple pregnancy rate was 15.4% (1 triplet and 21 twin pregnancies). The miscarriage rate was 22.4% (32/143).

Limitations, reasons for caution: The retrospective design of the study needs to be confirmed in larger and multicenter prospective studies comparing the strategy of vitrified donated oocytes and fresh ET with the policy of fresh donated oocyte and frozen/thawed ET.

Wider implications of the findings: The transnational oocyte donation program with vitrified oocytes is associated with good success rates. The number of inseminated oocytes represents a crucial factor for increasing the CPR, improving the embryo selection for fresh ET and giving more chances of pregnancy with the transfer of surplus vitrified embryos.

Trial registration number: Not applicable

P-232 A trophectoderm morphology can predict a live birth rate and gender imbalance

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Study question: Which morphology parameter is the most predictable in the live birth rate and can affect the sex ratio?

Summary answer: The trophectoderm grade (TE) can predict the live birth rate and skewed to male gender after single vitrified-warmed blastocyst transfers (SVBT).

What is known already: The Gardner and Schoolcraft grading system of blastocyst evaluation with morphology is the major predictor of the clinical outcome in ART. Inner cell mass (ICM) and trophectoderm (TE) morphology are strongly correlated between clinical pregnancy, live birth, and miscarriage. A greater degree of expansion of the transferred blastocysts showed a higher implantation rate. Therefore, it is essential to clarify which parameter is more predictable in clinical outcome during elective SVBT. However, SVBT has some potential limitations, including adverse effects such as a male-biased imbalance in the sex ratio.

Study design, size, duration: The retrospective analysis used 1138 cycles of SVBT in the Ojinmed IVF center, Mongolia, between May 2015 to January 2019. The morphology grade and blastocyst inner diameter compared with clinical pregnancy rate (CPR), live birth rate (LBR), and miscarriage. The sex ratio was estimated for all patients, excluding those who underwent PGT-A, donor oocytes, and monozygotic twins. Blastocyst quality was evaluated with Gardner and Schoolcraft grading system and measured inner diameter of the blastocyst.

Participants/materials, setting, methods: All patients underwent a clomiphene-based minimal ovarian stimulation protocol or drug-free natural cycle IVF treatment. On day 5 to 6, blastocysts that reached an inner diameter >160μm were immediately vitrified. Blastocyst morphology evaluated by ICM and TE grade. The CPR (with a confirmed gestational sac at 6-7 weeks of pregnancy) and the LBR (live birth at 22 weeks of pregnancy over) were estimated per embryo transfer procedure, followed by miscarriage rate.

Main results and the role of chance: The CPR was 44.69%, 38.97%, and 25.91% for A, B, C grades of ICM, respectively. And the LBR was 39.82 %, 34.62 %, and 19.1 % for A, B, C grades of ICM, respectively. TE was strongly related to CPR (aOR=2.47, 95% CI 1.71-3.58, p<0.01) and LBR (aOR=1.77, 95% CI 1.06-2.96, p=0.028) in univariate and multivariable logistic regression analysis (A grade vs C grade). Also, CPR and LBR were increased with blastocyst inner diameter, proportionally. The A and B grade ICM blastocysts showed 2.8 - 2.9 times less miscarriage rate than the C grade of ICM in the univariate logistic regression analysis. The result of multivariable logistic regression analysis showed B grade of ICM had 2.3 times less than C grade of ICM (aOR 2.36, CI 95% 1.20-4.61, p=0.012) and TE, patient age and blastocyst inner diameter were not significantly associated with miscarriage rate. The gender ratio was 56.8% (204/359) for male. The result of multivariable logistic regression analysis showed that A grade TE had a 2.3 times higher probability of male than C grade (aOR 2.31, CI 95% 1.22-4.37, p=0.01). Neither fertilization method, ICM, expiation grade, nor fertility case was significantly associated with the sex ratio.
Limitations, reasons for caution: The result of the current retrospective study is limited to data from a single IVF center.
Wider implications of the findings: Our study suggests that TE grade is the most predictable and ICM grade was associated with miscarriage. The high grade such as A-grade TE blastocyst transfer has more live birth rate, whereas it can affects at sex ratio in favor of male embryos after SVBT.
Trial registration number: not applicable

P-233 The spatial arrangement of blastomeres and time of cavitation forming as predictors of blastocyst quality

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Study question: Does the spatial arrangement of blastomeres and the start of blastulation affect blastocyst quality?
Summary answer: Better blastocyst quality is associated with the spatial arrangement of the embryo and the shorter time frame of blastulation (cavitation).
What is known already: The ability to select the human embryo with the highest implantation potential remains one of the greatest challenges in the management of In Vitro Fertilization patients. Several publications have proposed that additional morphological evaluations of blastomere arrangement and the dynamics of late-stage embryonic divisions might be a useful non-invasive way for embryo selection. In the last decade, the introduction of time-lapse technology enables continuous monitoring of embryo development, which leads to better outcomes than a selection based on the traditional morphology assessment**.**
Study design, size, duration: The spatial arrangement was defined as tetrahedrally if the cleavage planes were perpendicularly orientated, while embryos with rather parallelly orientated cleavage axes were considered as non-tetrahedral embryos. The injection time of ICSI was designated as "time zero" (t0), and EmbryoViewer software was used to calculate the time duration between injection and start of blastulation (cavitation). Obtained results were later correlated with the embryo's capability to form a blastocyst as well as with blastocyst quality.
Participants/materials, setting, methods: A total of 195 oocytes from 40 patients undergoing the antagonist cycle for ICSI treatment were evaluated. All blastocysts were cultured in Embryoscope™ according to the manufacturer's specifications (Vitrolife, Sweden). The Gardner and Schoolcraft scoring system was used to describe blastocyst quality. Statistical analyses were performed using IBM SPSS version 24. Data were reported as median and range. Differences between groups were tested using the Mann-Whitney U test. Statistical significance was defined as p<0.05.
Main results and the role of chance: Obtained data showed that 83.6 % (61/73) of embryos with tetrahedral arrangement formed blastocysts compared to 42.4% (50/116) of embryos with the non-tetrahedral arrangement (p<0,001). Moreover, tetrahedral embryos more frequently formed good quality blastocyst compare to the non-tetrahedral [59% (36/61) vs 18 (9/50) % respectively; p<0,001]. In addition, we found that good quality blastocyst had a significantly shorter time frame between injection and blastulation start, compared with blastocysts which did not reach good quality [95.00h (84-118) vs 102h (77-121) respectively; p=0,006].
Limitations, reasons for caution: The limitation of the present study was that due to the double-embryo transfer correlation between those morphokinetic parameters and pregnancy rate can not be calculated. Further research should link these morphokinetic parameters with pregnancy rate and live birth rate as well.
Wider implications of the findings: The potential of our findings is considerable, especially for countries with strict Embryo Law Regulation. Obtained results might be highly useful for selecting embryos with high implantation potential. In addition, the present work illustrates the possibility of additional information that can potentially be incorporated into an embryo classification model.
Trial registration number: not applicable

P-234 Doubling oocytes using the 1st polar body: how it works according to patient's age

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Study question: Does the 1st polar body (PB) transfer technique for oocytes and embryo doubling works at any patients' age?
Summary answer: The transfer of the patient's 1stPB to a donor oocyte shows an increase in the number of blastocysts in all studied age groups of patients.
What is known already: The problem of low ovarian reserve is one of the most difficult to solve by ART. Particular difficulties arise when patients are not ready to use donor oocytes. But transfer of the patient's 1st polar body (PB) to donor oocyte cytoplasm has proven to be an effective method to help patients with low ovarian reserve and poor response. In confirmation of this, three healthy babies have been born to this date thanks to this technique. But the question remains open as until what age the application of this method is effective. It was evaluated in this study.
Study design, size, duration: The study was performed in the Medical Center IGR from March 2017 to January 2021 and involved 695 cells: 361 oocytes (group A) obtained from 85 patients (mean age 38.4±6.4 years) and 334 oocytes (group B) that were obtained received from 47 donors (mean age 27.0±3.6). We evaluated the number of high quality blastocysts (HQB) from maternal and modified oocytes by the 1st PB transfer and blastocysts euploidy in the different age groups.
Participants/materials, setting, methods: We used patients' oocytes obtained from 85 women with low ovarian reserve and poor response. Donor oocytes have been previously enucleated and modified by the transfer of patients' 1stPB with further fertilization. The procedure was carried out using Nikon Ti Eclipse(Japan) inverted microscope, Saturn 3 laser console(UK). Preimplantation genetic testing for aneuploidy was performed using trophectoderm biopsy. Samples were diagnosed using Ion S5(USA). Statistical analysis was carried out using Chi-square test and Fisher's exact test.
Main results and the role of chance: In the group A there were 93 HQB (25.8%) that formed from 361 original patients' oocytes and 70 HQB (20.9%) developed from 334 modified oocytes (group B) on the post-fertilization day 5 or 6. The statistically significant difference (SSD) was not found between the groups on this parameter (p>0.05).

Also, the data were grouped according to the age of the patients, namely group I (23-30 years) that included 13 patients, group II (31-39 years) – 28 patients, group III (40-48 years) – 44 patients. In group I there were 12 HQB (25.0%) that formed from 48 original patients' oocytes and 14 HQB (29.9%) developed from 47 modified oocytes, in group II - 50 HQB (38.5%) that formed from 130 original patients' oocytes and 31 HQB (27.2%) developed from 114 modified oocytes, in group III - 31 HQB (16.9%) that formed from 183 original patients' oocytes and 25 HQB (14.5%) developed from 173 modified oocytes. The analysis showed SSD between the number of patients' blastocysts in groups II and III (p<0.001), as well as between the number of blastocysts from modified oocytes in groups II and III (p<0.005), I and III (p<0.05). There was not SSD among the number of euploid embryos.

Limitations, reasons for caution: With increasing age of patients, the morphological quality of their oocytes deteriorates, which is reflected in the structure of the polar bodies. In turn, the quality of the patient's polar body plays a major role in the successful modification of the donor's oocyte.
Wider implications of the findings: : Results of analysis shows that even in the group of advanced maternal age it is possible to increase the yield of high qualitative blastocysts at least by 14,5% using the1st PB transfer technique. At the same time, the biological relatedness of patient with the future child is maintaining.
Trial registration number: not applicable

P-235 Are morphokinetics parameters and profitability of an assisted reproduction cycle related to sperm selection technique? Microfluidic sperm sorter chip versus magnetic activated cell sorting

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Study question: Does microfluidic sperm sorter offer any biological improvement over magnetic activated cell sorting (MACS)?
Summary answer: Microfluidic approach for selecting high profile spermatozoa is as good as magnetic activated cell sorting in terms of morphokinetic, fertilization and good quality blastocyst rate.
What is known already: Microfluidic sperm sorter chip is a method for select non-fragmented DNA sperm. We know that the use of these non-fragmented DNA sperms can improve the clinical results and the useful blastocyst rate. As several studies have shown previously, embryos morphokinetics parameters are affected by culture medium, ovarian stimulation, oxygen tension, origin of the oocytes, or the age of the patient, this is why we wanted to compare if the cleavage times using time lapse technology, are different depending on the sperm selection method used to select sperm with the non-fragmented DNA.
Study design, size, duration: Prospective and observational study performed between May 2019 to January 2021 in IVI Madrid and IVI Sevilla. Seminal samples from couples participating in the study were divided into two aliquots; each of them was processed according to one of the study methods. 53 couples were included in the study. Half of the oocyte from each donor were microinjected with sperm selected through MACS (n=281) and the other half through a microfluidic device (n=275).
Participants/materials, setting, methods: These oocytes were microinjected with both types of sperm samples and incubated in EmbryoScope. Cellular events studied in this study included cellular divisions until blastocyst stage, appearance and fading of some cellular structures and the duration of the first, second and third cellular cycle (cc1, cc2 and cc3) as well as their synchrony (S1, S2, S3). Data were exported from the EmbryoViewer data base. We perform an ANOVA statistical analysis to analyze the data.
Main results and the role of chance: No significant differences between both sperm selection methods were found regarding the time of cell division (from T2 to Tblstocyst), the cellular cycles duration (cc1, cc2 and cc3) or the synchrony of the cellular cycles divisions (s1, s2 and s3). However, a clear trend towards statistical significance has been found in both duration of cc2 (p=0.052) being longer in MACS embryos than in microfluidic sperm sorting embryos, and in the expansion of the blastocyst, which occurs earlier in embryos that come from MACS than in those that come from microfluidic sperm sorting (p=0.097). These two events could indicate a better embryo cleavage dynamic in the case of MACS embryos, with a better blastocyst expandability and the necessary time to carry out all the biological events that must occur in the cc2.

However, significant difference was found in the direct cleavage from 1 to 3 cells embryo stage, which is one of the adverse events that more affects embryo implantation, being higher in microfluidic sperm sorting group (p=0,037). Finally, the fertilization rate (73.1% vs 76.9%) and the good quality blastocyst rate (53.7% vs 56.5%) were higher in MACS embryos than in microfluidic sperm sorting embryos, although no significant differences were found.
Limitations, reasons for caution: This study has been performed in donated oocytes, so these results may not be extrapolated to other groups of assisted reproduction patients. However, more data are needed to draw firm conclusions. Furthermore, it´s crucial to increase the sample size to check if the trends founded reach statistical significance.
Wider implications of the findings: Microfluidic sorting of unprocessed semen in un unselected population is as efficacious as magnetic activated cell sorting according embryo morphokinetic, fertilization rate and useful blastocyst rate. Microfluidic sperm sorting does not show clinical advantage over MACS considering this data collection.
Trial registration number: NCT04061484

P-236 The evaluation of apoptosis and luteinization process in cumulus cell culture of IVF patients in terms of embryo development and pregnancy

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Study question: Is there any relationship between apoptosis rate and progesterone levels in terms of the degree of embryo quality and clinical pregnancy success?
Summary answer: The cumulus cell apoptosis and progesterone levels measured at the hCG day were associated with embryo quality and clinical pregnancy success.
What is known already: The high rate of apoptosis at cumulus cells led to poor embryo development with the clinical pregnancy failure.
Study design, size, duration: It is prospective study carried out with 40 healthy women diagnosed as the male factor between September 2017 and April 2018.
Participants/materials, setting, methods: 40 healthy women aged between 27-38 and diagnosed as the male factor who were undergoing IVF participated the study. The cumulus cells were taken after denudation and cultured. The rate of the apoptosis were measured using with TUNEL method every 24 hours for 96 hours. In concominant with the apoptosis evaluation, the embryo progession and clinical pregnancy rate were also monitored. Progesterone levels taken at the hCG day were also measured.
Main results and the role of chance: In this study, it was shown that cumulus cell apoptosis was lower in the pregnant group compared to the non-pregnant group (p = 0.023), and progesterone value on the hCG day was higher in non-pregnant group (p = 0.021). From the data obtained at the end of the 4th day of culture showed that the apoptosis rate showed a higher tendency in non-pregnant women and this was statistically significant (p = 0.009). In our study, it was found that the ratio of apoptotic cells up to the 4th day showed a positive correlation with the progesterone levels on the hCG day (p = 0.001).

Briefly, these results were obtainedfrom this current study that 1) The increase in the rate of apoptotic cells of cumulus cell culture has a negative effect on pregnancy rates, 2) The progesterone levels measured on the day of hCG has a significant negative effect on pregnancy, 3) The increase in progesterone levels acts as a messenger about the apoptosis rate of cumulus cells, 4) The luteinization is strictly associated with the apoptosis of cumulus cells. Limitations, reasons for caution: This was a single center study. Results need to be validated across different centers and high numbered different study population.
Wider implications of the findings: The increase in progesterone value acts as a messenger about the rate of apoptosis of cumulus cells may be the prominent indicator to obtain high grade embryo with high clinical pregnancy success.
Trial registration number: 2017/5-8

P-237 The good, the fast and the slow: which is better? Live birth rate following single blastocyst frozen embryo transfer

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Study question: Are embryo quality and day of vitrification (Day 5, 6 or 7) associated with live birth rates (LBR) following single blastocyst transfer (SBT) in frozen embryo transfer cycle (FET)?
Summary answer: Both blastocyst quality and day of vitrification are significantly associated with LBRs, with very low LBR when poor quality embryos are frozen on day 6.
What is known already: Evidence suggests that chromosomal status (ploidy) is strongly associated with blastocyst morphology and good quality embryos are more likely to be euploid. Furthermore, previous studies have shown a relationship between the time that embryos need to reach blastocyst stage and their euploidy rate with slowly developing blastocysts showing higher rate of aneuploidy. Nonetheless, despite all this evidence little is known about the actual effect of the combination of blastocyst quality and day of its vitrification. The scope of this study was to quantify the actual effect of the embryo quality and day of vitrification on live birth rates following FET.
Study design, size, duration: Retrospective analysis of 1546 FET cycles with SBT conducted between 2017 and 2019 in the university-affiliated private clinic. The embryos used for FET were obtained from IVF/ICSI: with PGT (FET-PGT) or without PGT (FET0) or from donated oocytes (FET-DON).
Participants/materials, setting, methods: FET with natural, natural-modified and completely medicated cycles to prepare endometrial lining were included.

Blastocysts were classified according to Spanish Association for the Study of Reproductive Biology (ASEBIR) classification, ranging from A (the highest) to D (the lowest).

The impact on LBR of different subgroups, formed within FET-PGT, FET0, FET-DON groups due to different day of vitrification and blastocyst quality, was assessed, using logistic regression after adjusting for age, day of vitrification and embryo quality**.**
Main results and the role of chance: We included 1546 FET cycles. Of those, 543 (35%) corresponded to FET-PGT; 648 (42%) to FET0 and 355 (23%) to FET-DON cycles.

Overall, 1051 (68%) embryos were frozen on day 5(D5), 472 (30.5%) on day 6(D6) and 23 (1.5%) on day 7(D7). As far as embryo quality was concerned, 215 (13.9%) grade A; 957 (61.9%) B; 371(24%) C and 3(0.2%) D blastocysts were transferred.

LBRs were significantly different between different embryos frozen on D5 44.3%; on D6 28.8% and on D7 8.7%, p<0.001. When blastocyst quality was considered, LBR were 48.4% for grade A; 42.5% for B; 25.1% for C and 0% for D, p<0.001.

After applying logistic regression analysis, the odds ratio (OR) for transferring D6-blastocyst was 1.08, 95% CI[0.45; 2.62] and blastocyst with grade B and C; 0.71, 95% CI[0.51; 1.00]; 0.57,95% CI[0.36; 0.88] respectively. However, after transferring D6-blastocyst graded as C, the OR was 0.33, 95% CI[0.12; 0.90].

Our predictive model showed that the impact of the embryo quality on LBR was sustained across three groups. Transfer of D5/D6 grade A blastocyst resulted in the highest, while D6-C in the lowest LBR in all the groups. In the latter case vitrification on D6 impaired additionally the outcome.
Limitations, reasons for caution: The study should be interpreted with caution due to its retrospective character and the assessment of blastocyst quality on the day of vitrification and not on the day its transfer.
Wider implications of the findings: Our robust findings could be considered a useful tool for counselling couples who seek advice regarding their expected success rates in the setting of FET with SBT. The very low livebirth rates in low quality (C) slow developing (D6) embryos should be communicated to patients prior to planning a FET.
Trial registration number: not applicable

P-238 Impaired oocyte fertilization is associated with a proinflammatory M1 phenotype of macrophages and the activation of the NLRC4 inflammasome

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Study question: Are inflammatory signatures in the blood of clinically asymptomatic patients predictive for oocyte fertilization success in ART treatment?
Summary answer: The proinflammatory M1 phenotype of macrophages combined with increasing numbers of cytotoxic T-cells and an upregulated inflammasome NLRC4 is associated with impaired oocyte fertilization.
What is known already: Oocyte fertilization is an indispensable step towards embryogenesis and reproductive success. Patients with acute or chronic inflammation are at risk of reduced fertilization rates and failure of assisted reproductive technology (ART) treatment. Inflammasomes are intracellular multiprotein complexes that mediate inflammatory tissue remodeling. They can be activated by endogenous and exogenous stimuli and result in maturation of the cytokines IL-1 and IL-18. Appropriate inflammasome activation serves for pathogen defense and tissue damage repair, while aberrant activation of inflammasomes can enhance uncontrolled tissue damage.
Study design, size, duration: This a prospective study including 39 patients stratified by fertilization rate (cut off 66%) of mature M II oocytes after ICSI procedure, compares 20 patients with high (mean 84,8%, range 67 -100 %) versus 19 patients with low (mean 29.4%, range 0-65%) rates.
Participants/materials, setting, methods: We performed FACS analysis of immune cell composition (leukocytes, neutrophils, monocytes, macrophage types M1 and M2, cytotoxic T-cells, T-helper cells) and utilized RT-qPCR analysis of three inflammasomes (AIM 2, NLRP3 and NLRC4) and their down-streaming proteins caspase 1 and IL-1ß. We focused on an initial and a late state during controlled ovarian stimulation (COS) procedure.
Main results and the role of chance: This study reports a cellular and molecular inflammatory signature associated with reduced oocyte fertilization rates after ICSI treatment in clinically asymptomatic patients.

On a cellular level proinflammatory M1 macrophages were significantly elevated in the low fertilization group ($p < 0.01$), additionally the ratio of proinflammatory M1 macrophages to anti-inflammatory M2 phenotype was inversely associated with oocyte fertilization rate ($p<0.001$). Cytotoxic T cells ($p < 0.05$) and the inflammasome NLRC4 ($p<0.01$) revealed identical patterns of association with fertilization rates in the group comparison.

In line with this pattern, there was a significant upregulation of Caspase 1 with a consecutive increase in IL-1ß activity in women with low fertilization rates ($p<0.05$).

No significant association was shown for the other tested immune cells (leukocytes, neutrophils, monocytes, T-helper cells) and inflammasomes (NLRC3, AIM2).
Limitations, reasons for caution: The preliminary results in this small study indicate an activation of the inflammasome NLRC4. This, however, has to be assessed on the protein level in a larger group of patients.
Wider implications of the findings: Systemic macrophage augmentation of M1 phenotype and cytotoxic T cell presence in combination with upregulated inflammasome NLRC4 are associated with impaired oocyte fertilization. The predictive information of the identified immunologic signature could indicate targeted treatment to down regulate systemic inflammation and the use of oocyte activators in the ART laboratory.
Trial registration number: none

P-239 In vitro maturation of human oocytes: a systematic review and data analysis

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Study question: Based on published studies, how effective is *in vitro* maturation (IVM) in different patient groups, and how does the maturation rate correlate with culture conditions?

Summary answer: Clinical IVM is most effective when patients receive only hCG trigger prior to oocyte collection. Multiple additional parameters influencing the outcome were identified.

What is known already: Despite being used for more than fifty years, the overall efficacy of human IVM has not yet been determined, and results are often conflicting. Indeed, IVM is still perceived skeptically by many embryologists and doctors and not widely used in clinical practice. This review aims to collect all available data in the literature regarding the efficacy of IVM analyzing characteristics of patients, treatment, or laboratory conditions that may influence the MII-rate (MR). Study design, size, duration: A systematic search was performed in the PubMed database following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The search was limited to studies in the English language published before October 2020 using the following keyword: "oocyte *in vitro* maturation».

Participants/materials, setting, methods: Inclusion criteria for studies were: reporting data obtained on immature human oocytes, which transitioned to the MII stage after IVM. The requirement was that the numbers of cultured and matured oocytes were reported. If available, additional data were collected including patients' characteristics (for example PCOS), hormonal stimulation prior to the procedure (administration of some FSH or hCG trigger or both), oocyte freezing before or after IVM, type of culture medium and supplements, etc.

Main results and the role of chance: A total of 350 publications were selected from 6866 search results, 436 abstracts, and 422 full read articles. Selected studies cover 21153 patients and 157420 immature oocytes cultured. It has been demonstrated that oocytes collected *in vivo* from adult, non-PCOS patients, who received only hCG trigger prior to the procedure had a statistically higher MII rate (66%) than oocytes from patients who received no gonadotropins or some FSH, or a combination of some FSH and hCG trigger (59%, 60% and 58% respectively). The same was valid for PCOS patients: MR in the trigger only cohort (66%) was significantly different from other cohorts. MR for *in vivo* collected oocytes (61%) from adult non-PCOS patients was significantly different from *ex vivo* collected oocytes (33%). MI stage oocytes at the moment of collection matured with a statistically higher rate (N=4322, 73%), than GV oocytes (N=3328, 54%). When *in vitro* matured oocytes were vitrified, their average survival rate was 81% (data from 50 studies on 1701 oocytes). Additionally, immature oocytes survived vitrification with a 75% rate (data from 30 studies on 4457 oocytes). Overall, ICSI fertilization rate for IVM oocytes was 69% (N=59914). A total of 747 babies born from IVM were reported.

Limitations, reasons for caution: Among selected publications only 2 were randomized controlled trials and therefore the main challenge of this review is striking differences in setups among included studies. However, despite not being a meta-analysis, this study calculated MR for the most frequent treatment modalities and additional individual factors, which might influence MR.

Wider implications of the findings: This review provides data regarding IVM efficiency in different cohorts of patients, performed under different culture conditions. Additional laboratory parameters influencing MR have been identified. Based on this new data, target groups benefiting the most were identified, and prognosis regarding the success of their treatment with IVM might be estimated.

Trial registration number: n/a

P-240 Human extracellular vesicles (EVs) secreted by aneuploid embryos potentiate development of non-invasive PGT-A RNA biomarkers and stimulate MUC1 up-regulation in primary endometrial stromal cells (ESCs)

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Study question: Could EVs secreted by aneuploid embryos a) serve for development of RNA biomarkers for PGT-A and b) elicit a relevant transcriptomic response in decidualised ESCs?

Summary answer: Aneuploid embryo EVs a) contain *PPM1J*, *LINC00561*, ANKRD34C and TMED10 in differential abundance from euploid EVs and b) induce up-regulation of *MUC1* in decidualised ESCs.

What is known already: Embryo aneuploidy accounts for approximately 50% of all recurrent implantation failures in women >35 years old. PGT-A identifies euploid embryos to increase implantation probability but the technology is controversial as it requires an invasive embryo biopsy with an elusive long-term biosafety. The development of non-invasive methods to screen out aneuploid embryos is paramount. It is also critical to decode the embryo-endometrial dialog underlying implantation failure. We have previously reported that IVF embryos secrete EVs that can be internalised by ESCs, conceptualising that successful implantation to the endometrium is facilitated by EVs, which may additionally serve as biomarkers of ploidy status.

Study design, size, duration: Embryos destined for biopsy on days 5-7 for PGT-A were grown under standard conditions. Spent media (30µl) were collected from euploid (n=175) and aneuploid embryos (n=145) at both cleavage (days 1-3) and blastocyst (days 3-5) stage. Media samples from n=35 cleavage embryos were pooled in order to obtain five euploid and four aneuploidy pools. Blastocyst media were pooled to create one euploid and one aneuploid pool. ESCs were obtained from five women undergoing diagnostic laparoscopy.

Participants/materials, setting, methods: The study was realised at a research hospital. EVs were isolated from euploid and aneuploid Day3 pools with differential ultracentrifugation and EV-RNA sequencing was performed following the SMARTer Stranded Total RNA-Seq approach. ESCs were decidualised (E2:10nM, P4:1uM, cAMP:0.5 mM twice every 48 hours) and treated for 24 hours with 50 ng/ml euploid or aneuploid EVs extracted from blastocyst media. RNA sequencing was performed on ESCs following the Truseq RNAseq protocol.

Main results and the role of chance: Aneuploid cleavage stage embryos (n=4) secreted EVs that were less abundant in RNA fragments originating from the genes *PPM1J* (log2fc=-5.13, p=0.011), *LINC00561* (log2fc=-7.87, p=0.010) and *ANKRD34C* (log2fc=-7.30, p=0.017) and more abundant in *TMED10* (log2fc=1.63 p=0.025) compared to EVs (n=5) from euploid embryos. Decidualisation *per se* induced downregulation of *MUC1* (log2FC=-0.54, p=0.0028) in ESCs as prerequisite for the establishment of receptive endometrium. The expression of *MUC1* transcript in decidualised ESCs was significantly increased following treatment with aneuploid compared to euploid embryo-secreted EVs (log2FC=0.85, p=0.0201).

Limitations, reasons for caution: The findings of the study may require validation utilising a second cohort of EVs samples.

Wider implications of the findings: This discovery that the RNA cargo of EVs secreted from aneuploid cleavage stage embryos is diverse from that of euploid embryos potentiates the development of non-invasive methodology for PGT-A. The upregulation of *MUC1* in decidualised ESCs following aneuploid embryo EV treatment proposes a new mechanism underlying implantation failure.

Trial registration number: NA

P-241 Construction of a Machine Learning algorithm based on early morphokinetics for human blastocyst development prediction: a retrospective analysis of 575 cleavage-stage embryos

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Study question: Can morphokinetic features included into Machine Learning (ML) algorithms identify cleavage-stage embryos with the best chance to reach the expanded blastocyst stage on day 5?

Summary answer: A ML algorithm based on early morphokinetic features can identify cleaving embryos that will reach the expanded blastocyst stage on day 5.

What is known already: To date, the conventional morphology assessment of cleaving human embryos has a limited predictive power on further embryo developmental potential. The morphokinetic analysis using Time-Lapse systems (TLS) was introduced in order to provide a new tool to identify dynamic

biomarkers of embryo quality. More recently, ML approach has been applied for the analysis of specific embryo-related features, aiming at developing predictive algorithms to assess the embryo development potential.
Study design, size, duration: We retrospectively analysed 575 embryos obtained from 80 women aged 25-42 years, with normal BMI, AFC≥8, day 3 FSH<12 IU/l, AMH>2.5 ng/ml, no diagnosis of polycystic ovary syndrome or endometriosis. These patients underwent IVF at our IVF Unit between March 2018 and March 2020; their embryos were cultured using the Geri plus® TLS and a single blastocyst transfer was performed.
Participants/materials, setting, methods: A total number of 29 morphological and morphokinetic parameters were considered to build six different ML algorithms. The performance to assess which was the best-fitting algorithm was calculated using the ROC curve considering accuracy (% of embryos correctly classified by the algorithm), Cohen-kappa coefficient (measurement of the agreement among features), mean number of TP (embryos correctly classified as undergoing developmental arrest), mean number of TN (embryos uncorrectly classified as undergoing developmental arrest).
Main results and the role of chance: Overall, 210 embryos progressed to the expanded blastocyst stage on day 5 (BL group), whereas 365 displayed developmental delay or arrest at any stage (nBL group). Among the six different algorithms, the best-fitting algorithm was obtained using the *Kbest* features selection approach combined with a *Random Forrest* evaluation strategy. This algorithm was based on 7 variables: embryo morphological score on day 2, pronuclear fading time (tPNf), completion time of cleavage to two, four and eight cells (t2, t4, and t8 respectively), time intervals t4-t3 and t8-t4. The algorithm showed an AUC of 0.78, with an accuracy of 0.73, a Cohen-kappa of 0.41, a mean TP number of 302/365 embryos in the nBL group and a mean TN number of 120/210 embryos in the BL group. Mean false positive (FP) and false negative (FN) numbers were of 63 and 90.2, respectively.
Limitations, reasons for caution: The results obtained in this study may not be generalizable to patients with other clinical characteristics, to other time-lapse systems or different laboratory settings. The predictive power of the algorithm should be validated prospectively on a larger number of embryos.
Wider implications of the findings: The current study represents a preliminary analysis for the development of hierarchical predictive models for embryo assessment based on their developmental potential, that embryologists will be able to apply as a support for decision-making.
Trial registration number: Not applicable

P-242 Gene expression profiles of SARS-CoV-2-associated receptors and proteases in human early embryonic development and follicular cells

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Study question: Are the oocytes, embryos, granulosa and cumulus cells, used during ART, susceptible to the SARS-CoV-2 infection?
Summary answer: Transcriptomic analyses of SARS-CoV-2-associated receptors and proteases strongly suggest that blastocysts are most permissive to SARS-CoV-2 compared with mature oocytes and day 3 embryos.
What is known already: Very few studies analyzed the gene expression profiles of SARS-CoV-2-associated receptors and proteases, mainly focusing on *ACE2* and *TMPRSS2* expression, resulting in partial knowledge in different specimens from female genital tract. To date, the gene expression profile of SARS-CoV-2 host entry candidates in the entire preimplantation embryos is scarcely available. Moreover, reports on oocyte and granulosa cells susceptibilityto SARS-CoV-2 are very sparse.
Study design, size, duration: To address this question, we retrospectively examined the gene expression profiles of SARS-CoV-2-associated receptors and proteases in human granulosa cells (GCs), cumulus cells (CCs), mature oocytes, day 3 embryos, blastocysts and trophectoderm cells obtained from our previously described Affymetrix microarray data.
Participants/materials, setting, methods: Human GCs and CCs (n=17), mature oocytes (n=6), and preimplantation embryos (n=20) were analyzed. The comparison of gene expression levels of receptors and proteases closely related to SARS-CoV-2 infection. For each gene, the number of samples with the probe set 'present', based on the detection call, was analyzed. Each probe set was classified according to the signal intensity value median, as low (<100), medium (100-200) or high expression level (>200).
Main results and the role of chance: *ACE2*, *BSG*, *CTSL*, *CTSA* were detectable at high expression level in all mature oocyte samples, while only *CTSL* was strongly expressed in all day 3 embryos. The most representative dual co-expression of SARS-CoV-2-associated receptor and protease (60% of samples) during the embryonic genome activation stage (EGA) was *ACE2-CTSL* and *BSG-CTSL*. In blastocysts, *ACE2*, *BSG*, *CTSL*, *CTSA* and *FURIN* were detectable in the entire cohort at high expression level, and the prevalence of the different dual co-expression of SARS-CoV-2-associated proteases and receptors was optimal (100% of samples). Interestingly, only *CTSL* was detectable in all trophectoderm samples and a prevalence of 60% was found for the *BSG-CTSL* co-expression. *ACE2*, *BSG*, *CTSL* and *CTSA* were present at high expression level in CCs samples. In contrast, *ACE2* and *BSG* expression was very low while *CTSL* and *CTSA* showed a high expression level in GCs.A prevalence of 100% was reported for *ACE2-CTSL*, *ACE2-CTSA* co-expression for both cell types. In addition, *BSG-CTSL* and *BSG-CTSA* co-expression were also present in all CCs against ~70% in GCs samples. This data suggest a potential risks of SARS-CoV-2 infection either GC or early embryo development.
Limitations, reasons for caution: Analyses of Affymetrix microarray gene expression data were performed in non-COVID-19 patients. Whether the SARS-CoV-2 infection change the gene expression profile of SARS-CoV-2-associated receptors and proteases is under investigation.
Wider implications of the findings: Specimens from female genital tract may be considered as potential targets for SARS-CoV-2.
Trial registration number: not applicable

P-243 Improving ERICA's (Embryo Ranking Intelligent Classification Assistant) performance. Should we train an AI to remain static or dynamic, adapting to specific conditions?

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Study question: Can ERICA's deep-learning capabilities allow it to learn specifics from individual clinics, and improve its performance through a quality assurance and fine-tuning process?
Summary answer: Quality assurance and fine-tuning allowed ERICA to adapt to unique specifications of individual clinics, resulting in an improved performance at each clinic.
What is known already: Machine learning (ML) solutions to real-life problems have shown that generalizability (applicability of a model to different scenarios) of a single model is fundamentally a suboptimal approach, due to the risk of underspecification. Under-specification becomes relevant in environments where there is a myriad of protocols and approaches, like during IVF treatments. It is naïve to assume that different features extracted from embryos to predict treatment success weigh the same along the overall heterogeneity of protocols. This underspecification problem takes special relevance when deploying an ML-based product, like ERICA, in a clinical setting.
Study design, size, duration: Retrospective analysis of results from the quality assurance (QA) and fine-tuning (adaptation) process performed for a deep learning algorithm named ERICA (Embryo Ranking Intelligent Classification Assistant) at five clinics (1879 embryos) between August and September 2020.
Participants/materials, setting, methods: QA and fine-tuning consist of a transfer-learning approach (of the ERICA Core model) and re-training using embryos of each clinic exclusively. Results are assessed by a 10-fold cross validation approach, which splits the database in 10 and iteratively validates on each by training on the rest. Performance of ERICA is assessed both before and after

the fine-tuning process and results are presented as averages per clinic. Embryos considered for QA and fine-tuning had known outcome.

Main results and the role of chance: After the fine-tuning, ERICA showed an average improvement of 13% in accuracy (from 50.2% to 63.2%); 36.6% in specificity (from 22.4% to 59%); 11% for Positive Predictive Value (from 51% to 62); 19.6% for Negative Predictive Value (from 44.6% to 64.2%), and 3.4% for F1 score (from 60% to 63.4%). Sensitivity decreased from 78% to 65.4%.

Our results suggest ERICA's Core is robust lending itself to be fine-tuned. It learns from individual laboratory specifics, and in this way adapts to new clinics. The results demonstrate that the Core model tends to classify embryos from new clinics as having a good prognosis, since it showed a high sensitivity and low specificity, both showing an improved balance following the fine-tune process. Additionally, the probability of finding a good prognosis embryo in the different labels, behaved as expected, decreasing its probability from Optimal (65.8%) to Poor prognosis (37.4%).

Limitations, reasons for caution: underspecification is a challenge to Artificial Intelligence (AI) based solutions pursuing a general model. For this study, our approach of QA followed by a fine-tuning process to overcome underspecification, was successful. However, it was only applied to 5 clinics, and the findings remain to be proven on a larger scale.

Wider implications of the findings: Performance of QA should be considered standard before clinical implementation of any AI based solution. Our results should be interpreted as the theoretical/expected future performance of ERICA for each clinic. Regular assessments on performance for all models generated after fine-tuning are encouraged.

Trial registration number: not applicable

P-244 ERICA's (Embryo Ranking Intelligent Classification Assistant) ranking, based on ploidy prediction, is strongly correlated with pregnancy outcomes

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Study question: How does ERICA perform when ranking the most suitable embryos for transfer in terms of clinical pregnancy, and the presence of a fetal heartbeat (FHB)?

Summary answer: ERICA's Artificial Intelligence ranking system was positively correlated with outcomes defined as implantation and presence of FHB. Best-ranking embryos outperformed lower-ranking embryos by statistical significance.

What is known already: ERICA, the Embryo Ranking Intelligent Classification Assistant, is a deep learning AI system trained to rank embryos based on their ploidy status, which is highly correlated with successful treatments.

ERICA ranks the embryos according to their prognosis predictions and labels them into four quality categories: optimal, good, fair, and poor. ERICA's performance in the clinic remains to be tested.

Study design, size, duration: Retrospective analysis on ERICA's performance over 4 consecutive months after quality assurance and fine-tuning processes. We compared both the ranking and prognosis of the AI algorithm against clinical outcomes in IVF cycles and subsequent embryo transfers. For this study, all cycles where ERICA was used to assist embryologists during the embryo selection process were included. Double embryo transfers with a single FHB where excluded.

Participants/materials, setting, methods: Total 77 cycles with 81 transfers of 98 embryos (17 cases underwent a double embryo transfer) from two IVF clinics. Evaluated clinical outcomes included biochemical pregnancy test (defined as beta human chorionic gonadotropin >20 mUI/ml), and presence/absence of FHB. We compared the ERICA rankings and predictions against outcome and a sub-analysis was performed on transferred embryos with known ploidy status (14 embryos).

Main results and the role of chance: The distribution of embryos within the ERICA categories are 42% for optimal, 38% for good, 19% for fair, and 6% for poor. The observed biochemical pregnancy rate was 51%, 25%, 47% and 33% respectively, and 39%, 22%, 42%, 17% for FHB. We found statistical significance (Z=1.78; p=0.0378) for the proportion of biochemical pregnancy between transfers labelled by ERICA as optimal (51%) and all lower rankings (33%). The proportion of transfers with presence of FHB within the optimal group was 39%, compared with 29% for the rest of the embryos. This did not show statistical significance (Z= 1.141; p=0.127). Additionally, we observed that the proportion of biochemical pregnancy and presence of FHB in the group of transfers with known ploidy (n=14) was 50% and 36% respectively, and the transfers with unknown ploidy and labelled as optimal by ERICA (n=35) was 54% and 43% respectively.

Limitations, reasons for caution: This is the first report on ERICA's performance on real clinical data, and despite being a relatively small dataset, we observed statistical significance of the embryos labelled by ERICA as having optimal quality. Further studies should be conducted with larger datasets and more clinics included to strengthen the evidence.

Wider implications of the findings: This is the first report on ERICA's performance on real clinical data, and despite being a relatively small dataset, we observed statistical significance of the embryos labelled by ERICA as having optimal quality. Further studies should be conducted with larger datasets and more clinics included to strengthen the evidence.

Trial registration number: not applicable

P-245 Machine learning predicting oocyte's fertilization and blastocyst potential based on morphological features

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Study question: Can machine learning (ML) predict oocyte's fertilization and blastocyst development potential based on morphological features extracted from single static images?

Summary answer: AI accurately predicted 70.4% of fertilization and 60.4% of blastocyst development outcomes from a database of 1000 oocytes.

What is known already: Some morphological features of the oocyte have been associated with IVF-related outcomes, such as size, shape, and coloration of zona pellucida, polar body, *perivitelline space*, cytoplasm, and the meiotic spindle. Based on these characteristics, clinics might discard the low-quality oocytes according to a subjective assessment. AI-based algorithms could reduce the subjectivity and improve prediction on IVF outcomes such as successful fertilization and blastocyst development.

Study design, size, duration: Non-intervention study based on a cohort of 1000 oocytes' micrographs collected between January 2019 and December 2020 from two IVF clinics. The inclusion criteria were known fertilization and blastocyst development outcome, and patient's age between 25 and 45 years old undergoing IVF/ICSI treatment. Different features were considered for this study including metadata from oocyte's (e.g. age, source), as well as manually extracted morphological features from the oocytes' images (e.g. diameters, shape, granularity, presence/absence of spindle).

Participants/materials, setting, methods: We trained three machine-learning (ML) classifiers (i.e. Support Vector Machine, logistic regression, and neural networks) to predict successful fertilization and blastocyst development. For the training process we used a 10-fold cross validation approach to assess the model's generalization capabilities. Besides we tested the statistical difference of each feature among groups (i.e. fertilized and no fertilized) using a two sided Student's t-test for numerical and Z-test for categorial features (significance of $p < 0.01$).

Main results and the role of chance: Our database showed 68.2% of successful fertilization and 34.8% of blastocyst formation. To balance the training data (50% per training class), we aleatory selected 318 and 348 samples per branch of successful/unsuccessful fertilization and blastocyst formation, respectively. From all ML classifiers, the neural network obtained the best results with an accuracy of 0.70 (AUC of 0.74) for predicting fertilization; and an accuracy of 0.60 (AUC of 0.62), for predicting blastocyst formation.

We found that spherical shape, presence of meiotic spindle, clear coloration, larger oocyte diameter, thicker zona pellucida, and smaller vacuoles are statistically associated with both successful outcomes.

As expected, we also found a strong association between age groups and outcome. The younger group (<35 years) demonstrated to have a larger proportion of successful fertilization compared to the rest of the age groups (36-37, 38-39, 40-42, >42). For the blastocyst formation we observed a similar association.

Limitations, reasons for caution: It is relevant to note that all cycles were performed under a mini-IVF protocol. Oocytes extracted through conventional stimulation might show the same associations, but it would need further testing.

Wider implications of the findings: The present study revealed that our system can predict fertilization success and blastocyst development potential based on metadata and morphometric features extracted from single digital micrographs of oocytes, offering a novel, adaptable and robust integration into clinical practice.

Trial registration number: CONBIOETICA-09-CEI-001-2017-0131

P-246 Oocyte degeneration after ICSI is not an indicator of live birth in young women

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Study question: To investigate whether oocyte degeneration after intracytoplasmic sperm injection (ICSI) is an indicator for predicting the cumulative live birth rate.

Summary answer: The presence of oocyte degeneration after ICSI is not an indicator for predicting the cumulative live birth rate per OPU cycle in young women.

What is known already: Oocyte degeneration may be associated with decreased embryo quality for embryo development kinetics was disturbed. No differences in clinical outcomes such as implantation rate or clinical pregnancy rate were found in fresh embryo transfer cycles in retrospective studies.

Study design, size, duration: This was a retrospective cohort study, including all the oocyte retrieval cycles from young women who underwent ICSI from January 2018 to December 2019 at the Reproductive Medicine Center of the First Affiliated Hospital of Sun Yat-sen University.

Participants/materials, setting, methods: The inclusion criteria were as follows: female age was younger than 35 years; the first or second oocyte retrieval cycles ;

[t]he number of oocyte retrieval was between 8 and 20; all the cycles performed fresh embryo transfer on day 3 after insemination. Cycles with at least one oocyte degenerated after ICSI were defined as the oocyte degeneration group (OD group), and cycles with no oocyte degenerated after ICSI were defined as the non-OD group.

Main results and the role of chance: There were no significant differences with regards to implantation rate (38.5% *vs* 35.1%, *P*=0.302), clinical pregnancy rate (54.9% *vs* 50.3%, *P*=0.340), and live birth rate per OPU cycle (47.0% *vs* 42.9%, *P*=0.395) between OD and non-OD groups. Initial gonadotropin dosage, E2 level on hCG day and the number of matured oocytes appeared to be independent risk factors for OD, after adjustment for female age, female BMI, duration of gonadotropin administration, FORT, number of retrieved oocytes and different technicians. The adjusted odds ratio of live birth rate per OPU cycle were similar in subgroups with different oocyte degeneration rates. The ongoing pregnancy/live birth rate per transfer in FET cycles was not significantly different between OD group and non-OD groups (38.8% *vs* 43.9%, *P*=0.439). The cumulative live birth rate per OPU cycle was also comparable between the OD group and non-OD group (63.4% *vs* 64.8%, *P*=0.760).

Limitations, reasons for caution: The time interval for the follow-up was not long enough for all the frozen embryos to be transferred. Moreover, the retrospective nature of the study introduces the potential to include confounding variables that may bias our results, although we performed multiple logistic regression analysis to minimize these effects.

Wider implications of the findings: The presence of oocyte degeneration is not an indicator for predicting the cumulative live birth rate per OPU cycle in young women. Initial gonadotropin dosage, E2 level on hCG day and the number of matured oocytes appeared to be independent risk factors for oocyte degeneration.

Trial registration number: none

P-247 Application of deep learning for automated measurement of key morphological features of human zygotes for IVF

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Study question: Can deep learning (DL) algorithms trained on time-lapse videos be used to detect and track the size and gender of pronuclei in developing human zygotes?

Summary answer: Our DL algorithm not only outperforms state-of-the-art models in detecting the pronuclei but can also accurately identify and track its gender and size over time.

What is known already: Recent researches have explored the use of DL to extract key morphological features of human embryos. Existing studies, however, focus either on blastocysts' morphological measurements (Au et al. 2020) or on embryos' general developmental stages classification (Gingold et al. 2018, Liu et al. 2019, Lau et al. 2019). So far, only one paper attempted to evaluate zygotes' morphological components but stopped short of identifying the existence and location of their pronuclei (Leahy et al. 2020). We address this research gap by training a DL model that can detect, classify the gender, and quantify the size of zygotes' pronuclei over time.

Study design, size, duration: A retrospective analysis using 91 fertilized oocytes from infertile patients undergoing IVF or ICSI treatment at Hanabusa Women's Clinic between January 2011 and August 2019 was conducted. Each embryo was time-lapse monitored using Vitrolife which records an image every 15 minutes at 7 focal planes. For our study, we used videos of the first 1-2 days of the embryo from its 3 central focal planes, corresponding to 70-150 images per focal plane.

Participants/materials, setting, methods: All 273 timelapse videos were split into 30,387 grayscale still images at a 15-minute interval. Each image was checked and annotated by experienced embryologists where every pixel of the image was classified into 3 categories: male pronuclei, female pronuclei, and others. Images were converted into grayscale, resized into 500x500 pixels, and then fed into a neural network with the Mask R-CNN architecture and a ResNet101 backbone to produce a pronuclei instance segmentation model.

Main results and the role of chance: The 91 embryos were split into training (~70% or 63 embryos) and validation (~30% or 28 embryos). Our pronuclei model takes as input a single image and outputs a bounding box, mask, category, confidence score, and size measured in terms of pixel for each detected candidate. For prediction, we run the model on the 3 middle focal planes and merge candidates by using the one with the highest confidence score. We used the mean-average precision (mAP) score to evaluate our model's ability to detect pronuclei and used the mean absolute percentage error (MAPE) between the actual size (as annotated by the embryologist) and the predicted one to check the model's performance in tracking the pronuclei's size.

The mAP for detecting pronuclei, regardless of its gender, achieved by our model was 0.698, higher than the 0.680 value reported in the Leahy et al. paper (2020). Breakdown by gender, our model's mAP for male and female pronuclei are 0.734 and 0.661 respectively.

The overall MAPE for tracking pronuclei's size is 21.8%. Breakdown by gender, our model's MAPE for male and female pronuclei are 19.4% and 24.3% respectively.

Limitations, reasons for caution: Samples were collected from one clinic with videos recorded from one time-lapse system which can limit our results' reproducibility. The accuracy of our DL model is also limited by the small number of embryos that we used.

Wider implications of the findings: Even with a limited training dataset, our results indicate that we can accurately detect and track the gender and the size of zygotes' pronuclei using time-lapse videos. In future models, we will increase our training dataset as well as include other time-lapse systems to improve our models' accuracy and reproducibility.

Trial registration number: not applicable

P-248 Statistical estimation for incidence of blastocyst trophectoderm vesicles (TVs) and efficacy of assisted hatching (AH)

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Study question: The aim of this study is to analyse the association between blastocyst diameter and TVs development, and to examine the efficacy of AH.
Summary answer: Blastocysts with a diameter of more than 170 µm leads to high incidence of TVs and AH applied from the incidence should be effective.
What is known already: TVs are protrusion of trophectoderm cells often observed in expanding blastocyst stages. TVs can be observed in expanding blastocysts regardless of Intracytoplasmic sperm injection (ICSI) and Conventional-IVF (C-IVF), when the internal pressure of blastocysts increase. The rate of TVs incidence in blastocysts inseminated by ICSI is higher than that by C-IVF, due to penetration of the needle into the zona pellucida. Moreover, it has been reported that TVs may inhibit blastocyst hatching. However, the developmental timing of TVs is still unclear, and there is no study that has analysed the association between blastocyst diameter and the incidence of TVs.
Study design, size, duration: 1) Diameters and TVs incidence of blastocysts by ICSI and C-IVF were measured, and the cut-off value and the area under the curve (AUC) of the receiver operating characteristic (ROC) curve were calculated to estimate the timing of TV incidence. 2) We analysed the clinical pregnancy rates of blastocysts with TVs treated by AH compared to those of blastocysts by C-IVF not subjected to AH.
Participants/materials, setting, methods: This study included 821 transferred frozen blastocysts ranging from March 2018 to November 2019. The embryos were cultured in a dry incubator after insemination by ICSI or C-IVF. Blastocyst freezing conditions were set at day5 to day7 with a diameter of more than 150 µm in inner diameter of zona pellucida, and this was measured before freezing. The ROC curve was performed using EZR statistical analysis software.
Main results and the role of chance: 1) The incidence of TVs in blastocysts by ICSI and C-IVF was 27.5% (117/424) and 14.6% (58/397) respectively. The rate of the incidence of TVs in blastocysts inseminated by ICSI and C-IVF; 8.6% (12/140) and 0.95% (1/105) in 150-159 µm, 12.7% (14/110) and 8.2% (6/73) in 160-169 µm, 40.6% (28/69) and 10.5% (6/57) in 170-179 µm, 55.6% (30/54) and 25.5% (13/51) in 180-189 µm, 66.7% (20/30) and 35.7% (10/28) in 190-199 µm, and 68.4% (13/19) and 26.8% (22/82) in the diameter of more than 200 µm. The cut-off value of the ROC curve was respectively 170 µm (sensitivity 78.6% and specificity 73.0%) and 176 µm (sensitivity 84.5% and specificity 59.6%) in the diameter; the AUC was 0.8 [95%CI:0.752-0.848] and 0.74 [95%CI:0.687-0.793] respectively. 2) The clinical pregnancy rate of TVs blastocyst vs C-IVF blastocyst was 52.7% (88/167) vs 57.8% (37/64) respectively. There is no significant difference between the two clinical pregnancy rates (P=0.556).
Limitations, reasons for caution: The findings of this study have to be seen in light of some limitations. Since this study aimed to analyse the incidence of TVs based on blastocyst size, we did not take into account the grade according to the Gardner classification and the number of trophectoderm cells.
Wider implications of the findings: Blastocysts inseminated by ICSI and C-IVF were highly likely to have TVs above 170 µm and 176 µm respectively. The clinical pregnancy rates of the blastocyst with TV treated by AH was similar to those of the C-IVF blastocyst.
Trial registration number: not applicable

P-249 Does the re-expansion of thawed embryos affect the clinical outcomes of human blastocyst vitrification?

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Study question: Is that essential for prolonged culture of thawed blastocysts in order to be fully re-expanded before transferring?
Summary answer: Ongoing pregnancy rates decreased in blastocysts that not fully re-expanded after thawing. What is known already: The thaw survival of blastocysts is examined based on morphology of inner cell mass (ICM) and trophectoderm (TE). However, thawed blastocysts experience multiple changes in morphology and might be collapse after thawing due to the presence of blastocoel cavity. It is then difficult to evaluate blastocyst quality. Therefore, the blastocyst re-expansion is considered as a criteria to assess quickly the competent embryos. It also reflects the status energy metabolism from high quality embryo. After all, there are still some controversial opinions about the influence of re-expansion status after thawing.
Study design, size, duration: This was a retrospective study based on data collected between October 2019 and December 2020. A total 528 thawed blastocysts which were divided into two groups according to the post-thaw reexpansion status: fully re-expanded blastocysts (n=416), partial or no re-expanded blastocysts (n= 112). The re-expansion status of blastocyst was assess prior to loading on the catheter by senior embryologists.
Participants/materials, setting, methods: Primary outcome is ongoing pregnancy. Only frozen single D5 transfer cycles were included. We excluded the frozen sperm/oocytes/embryos donation cycles, missing data, non-intact embryos after thawing. Statistical analyses were performed with T or chi-squared tests. Multivariable regression analysis was performed adjusting for the following confounding factors: age, BMI, embryo quality, re-expansion status, biopsied blastocyst.
Main results and the role of chance: Female age, BMI, number of previous cycles, endometrial thickness, positive HCG results, clinical pregnancy rate were comparable among patients within two groups. The rate of ongoing pregnancy rate in group 1 was significant higher compared with group 2 (51 vs 40.2, $p<0.05$). The number of good quality blastocyst transferred in group 1 was higher than in group 2 ($p<0.001$). However, under the same embryo quality, there were no difference between clinical pregnancy rate and ongoing pregnancy rate between two groups. When logistic regression were performed: only embryo quality, but not the re-expansion status, was noted to be an independent predictor of ongoing pregnancy (OR= 3.53;95% CI; 1.734-7.184;p=0.001).
Limitations, reasons for caution: The main limitation of the study is its retrospective design.
Wider implications of the findings: Clinical outcomes are comparable between re-expanded blastocyst and partial or no re-expanded blastocysts, although ongoing pregnancy can be improved when embryos are fully expanded. As expected, blastocysts quality has the most important impact on ongoing pregnancy rate.
Trial registration number: not applicable

P-250 Mineral oil with high viscosity improves the stability of pH and osmolality in the human in vitro culture system

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Study question: Do commercial mineral oil brands differ in their capacity to stabilize the human embryo culture system, and is this related to the oil's viscosity?
Summary answer: While the oils' viscosity only had minor effects on temperature maintenance, it showed a direct correlation with the stability of pH and osmolality during culture.
What is known already: Mineral oil is a key component of the *in vitro* embryo culture system, which stabilizes temperature, pH and osmolality of the media during culture. Its use has been implemented worldwide for several decades and many manufacturers currently produce and commercialize oil intended for human embryo culture. Unfortunately, oil remains as one of the less characterized products in the IVF laboratory due to a lack of standardized nomenclature, production and testing. With differing physico-chemical properties, such as viscosity, oils produced by various manufacturers could behave differently to the same culture conditions and, thus, its use may need to be adjusted accordingly.
Study design, size, duration: Viscosity was quantified in three high-viscosity (H-V) and three low-viscosity (L-V) oils with a viscosity-meter. The required time for media's pH to equilibrate using each oil was studied, as well as its subsequent stability outside the incubator for 30min. In-drop temperature was assessed during 15min when taking a dish outside the incubator, and again when putting it back. Additionally, each oil's capacity to avoid media evaporation was studied with daily osmolality measurements during 7 days.

Participants/materials, setting, methods: pH equilibration was measured with a continuous pHmeter (Log&Guard, Vitrolife) in 4-well dishes prepared with 600µl of medium and 500µl of oil. For the other experiments, 35mm dishes with 4ml of oil and 20µl media droplets were used. pH stability was assessed after 0, 15 and 30min outside the incubator with a blood-gas-analyzer (epoc,SiemensHelthineers). A fine-gauge thermocouple was used to measure in-drop temperature loss/recovery. Daily osmolality readings were taken with a vapor pressure osmometer (Vapro5600,Wescor).

Main results and the role of chance: The selected oil samples had a viscosity of 115, 111, 52, 22, 18, and 12cP. The medium's pH took approximately 12h to completely equilibrate under H-V oils, while it took less than 4h in L-V. Similarly, the rise in pH after 30min on a heated stage outside of the incubator with room atmosphere was 0.03, 0.04, 0.06, 0.13, 0.17, and 0.26, respectively.

Dishes were taken out of the incubator and placed on a heated surface. In the first five minutes, the in-drop temperature loss ranged between -0.22 and -0.13oC/min, with no significant differences observed between oil types. However, temperature plateaued at a significantly higher value in L-V oils (36.5oC), compared to H-V brands (36.25-36.1oC; p=0.0005). By contrast, all samples followed a similar pattern when the dishes were returned to the benchtop incubator, with temperature taking around 7 minutes to completely recover.

Some media evaporated in all oil groups during the 7-day culture in a dry benchtop incubator. The linear regression performed to compare the evaporation rate between groups showed a statistically significant correlation between oil viscosity and the rate of evaporation (p<0.0001), with an osmolality rise ranging between +2.55mmol/kg/day in the most viscous oil and +6.29mmol/kg/day in the least viscous.

Limitations, reasons for caution: While the selected oils for this study represent a wide range of options in the market, future projects could widen this selection and include additional tests, such as optimized bioassays. Results may vary between centers, and thus each laboratory should test and optimize their culture system with their own settings.

Wider implications of the findings: Different oil brands have shown differing physico-chemical properties that have a direct effect on the culture system and the stability of several culture conditions. These results may be of major importance to adapt the settings and methodologies followed in each IVF laboratory according to the type of oil being used.

Trial registration number: Not applicable

P-251 To collapse or not to collapse blastocysts before vitrification? A matched case-control study on single vitrified-warmed blastocyst transfers

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Study question: Does laser-induced artificial blastocoel collapse result in better blastocyst cryopreservation survival and higher live birth rate (LBR) in comparison with intact counterparts?

Summary answer: Compared to vitrification of intact blastocysts, collapsed blastocysts resulted in higher survival and for 5% higher LBR. Neonatal outcomes were comparable in both groups.

What is known already: Blastocysts have long been considered a stage that is suboptimal for freezing-thawing procedures due to their high fluid content and different cell types. The development of a modified vitrification technique has enabled blastocysts to better survive cryopreservation compared to a slow freezing procedure. Many studies on the optimization of cryopreservation of blastocysts have mentioned the need for artificial collapsing of the blastocoel prior to cryopreservation, thereby reducing the risk of intracellular ice-crystals formation. However, the effectiveness of artificial collapsing on blastocyst survival rate, single vitrified-warmed blastocyst transfer (SVBT) outcome and on safety of such intervention remains to be confirmed.

Study design, size, duration: A retrospective matched case-control study of transfers of single blastocysts being artificially collapsed (case) or intact (control) before vitrification. A sample size of 306 cycles in both arms was needed to achieve 80% power to detect a difference between the groups of 10% with *P*<0.05. Controls were matched to cases on a 1:1 ratio by female age, parity, fresh and frozen cycle protocol, blastocyst age and quality, getting 309 pairs of cases and controls.

Participants/materials, setting, methods: Artificial collapsing was introduced into clinical practice gradually. In fresh IVF cycles (performed in university clinic from 2012 until 2014) with supernumerary blastocysts, half of the blastocysts were randomly selected before vitrification for laser-induced artificial collapsing. The other half was vitrified in intact form. Only the first transfers of a single vitrified-warmed blastocyst (n=818) were included in the study. By matching, 309 pairs of collapsed (study) and intact (control) SVBTs were identified.

Main results and the role of chance: Both groups were comparable by their characteristics in indications, female age, type and length of ovarian hyperstimulation, insemination method in fresh cycle, protocol for warmed blastocyst transfer, blastocyst quality and day of blastocyst vitrification. Survival rates in case and control group ((309/316) 97.8% and (309/323) 95.7%; *P*=0.13) were comparable, but optimal survival rates (100% survival and re-expansion after warming) was significantly higher in artificial collapse group ((247/316) 78.2% and (225/323) 69.7%; *P*=0.01). Clinical pregnancy rates ((120/309) 38.8% and (110/309) 35.6%; *P*=0.4), miscarriage rates ((15/120) 12.5% and (24/110) 21.8%; *P*=0.06) and LBR per transfer ((100/309) 32.4% and (85/309) 27.5%; *P*=0.19) or LBR per warmed blastocyst ((100/316) 31.6% and (85/323) 26.3%; *P*=0.14) were not statistically different between case and control groups. Since the study was powered to detect a 10% difference, the possibility of type 2 error cannot be excluded. Perinatal outcomes were available for 175 live births. There were 10.5% (10/95) preterm births in the study group vs. 16.3% (13/80) in control group (*P*>0.05). Birth weights (3,308 g (SD 592 g) vs 3,308 g (SD 738 g) and sex ratio (50.7% vs 49.2% boys) were also comparable between both groups (*P*>0.05). There were no major malformations detected in the study population.

Limitations, reasons for caution: The research is retrospective, but the cycles from both groups were performed in the same time period. The groups were balanced according to all possible confounders. Blastocysts for vitrification were first categorized by quality groups and embryos from each category were randomized for collapsing or for remaining intact.

Wider implications of the findings: No significant difference was found in live births by this sample size. Nevertheless, increasing the success by 5% with the introduction of artificial collapsing can be an important step towards optimizing of blastocyst cryopreservation. To confirm a 5% improvement in results, a sample size of >2500 cases would be needed.

Trial registration number: The study has been approved by the National Ethics Committee of the Republic of Slovenia (0120-204/2016-2).

P-252 The correlation of first cleavage and blastulation timing to the euploid status of embryos after PGT-A

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Study question: To examine the correlation of first cleavage and blastulation timing on euploidy rates in IVF cycles after PGT-A.

Summary answer: The timing of blastulation is observed earlier in the euploid embryos.

What is known already: Embryo evaluation is one of the most critical processes that affect the clinical outcome in IVF cycles. Conventional morphologic assessment and morphokinetic assessment using time lapse technology are performed in order to select the embryo with the higher implantation potential to be transferred. It is stated that embryos with faster developmental potential, especially early forming blastocysts, show increased euploidy rate and higher implantation potential.

Study design, size, duration: This study includes ICSI/PGT-A treatments completed between May 2018 and December 2019. 117 blastocysts were biopsied and their euploidy status was analyzed by NGS. These embryos resulted from 32 different ICSI treatments. PGT-A was performed due to: a) repeated IVF failure, b) advanced maternal age, c) recurrent pregnancy loss.ICSI was implemented in all cases and blastocysts were vitrified awaiting the genetic results. Single euploid blastocyst transfer followed and clinical pregnancy rate was monitored.

Participants/materials, setting, methods: Based on the genetic results, the biopsied embryos were divided into two categories; group A representing the euploid embryos and group B the aneuploid embryos. The timing of 1st cleavage and the timing of blastulation, by means of forming a blastocoel, were investigated and compared between the two groups. The rate of early blastocysts in the two groups was also analysed. Early blastocysts are considered those formed at 96h ±2 of embryo culture post ICSI.

Main results and the role of chance: After the genetic analysis of the biopsied embryos, 37 blastocysts were included in group A-Euploid embryos and 80 blastocysts in group B-Aneuploid embryos. The mean time of the 1st cleavage division was similar between the two groups, with marginally no statistical significance (group A-euploid:25.9h, group B-aneuploid: 26.9h ,p>0.05). Regarding the blastulation time, it was achieved earlier in group A-Euploid, at a mean time of 102.6h, compared to the mean time of 106h in group B-Aneuploid (p<0.05). Between the cohort of the Euploid embryos (group A), there was a higher rate of early blastulating embryos, compared to the cohort of aneuploid embryos (Group B) (24% VS 17.5%), although it was not statistically significant (p>0.05). After transferring 1 euploid blastocyst, the ongoing pregnancy rate was monitored in 76.5%, independently of the 1stcleavage and blastulation time of the transferred embryo.

Limitations, reasons for caution: Further investigation in larger randomized studies is required, as only a limited number of cases were included in this study. Further analysis of the ongoing pregnancy rate between the euploid blastocysts, depending on other morphokinetic parameters would be of paramount significance, as well.

Wider implications of the findings: High clinical pregnancy rates observed independently of the analyzed time points, indicate high success rates obtained after PGT-A/NGS. Additionally, success rates show that trophectoderm biopsy is not hazardous for the embryo viability, if performed properly. Concluding, genetic testing combined with time-lapse microscopy may provide further information to improve IVF outcomes.

Trial registration number: N/A

P-253 Description of a rare spontaneous monozygotic blastocyst splitting into two discrete euploid blastocysts in vitro detected with time-lapse imaging and preimplantation genetic testing (PGT)

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Study question: Can spontaneous and complete blastocyst splitting into two, *in vitro,* be investigated using time-lapse imaging and biopsy of each trophectoderm, for inference of ploidy?

Summary answer: Time-lapse imaging combined with PGT-A gives insights into the incidence, dynamics and timing of rare blastocyst splitting and the ploidy status of each resulting blastocyst.

What is known already: It is well known that multiple births occur more often with Assisted Reproductive Technologies (ART) than spontaneous conception, even after single embryo transfer. The mechanism of Monozygotic Twinning (MZT) during ART is still unclear but cryopreservation, extended culture, PGT, maternal age and assisted hatching are reported risk factors. MZT is a rare phenomenon, with an incidence of 0.4% in natural conception compared with up to 4.9% in ART. The timing of embryo splitting dictates the type of twinning, in terms of chorionicity and amnionicty, and this is officially determined using ultrasound scanning.

Study design, size, duration: This is a case study describing the detection of the complete splitting of an IVF blastocyst at 140 hours post insemination (hpi), using time-lapse imaging.

The 40-year-old patient previously experienced biochemical pregnancy and several miscarriages; an ectopic molar pregnancy and a probable cornual ectopic. The 39-year-old male partner was normozoospermic.

Participants/materials, setting, methods: Facilitative laser breaching was performed, according to standard operating procedure, of the morula at 96hpi of embryo development, prior to PGT. Images were collected every 10 minutes and developmental events and embryos morphology annotated using the EmbryoScope+™ time lapse incubator and software.

Main results and the role of chance: Over 50,000 hatching blastocysts have been time-lapse imaged, scrutinised and annotated within this group of fertility clinics. This is the first time that such a rare blastocyst splitting event has been recorded and studied.

Following observation of two pronuclei following IVF and typical cleavage development to blastocyst, with facilitative zona breaching on, at 106.7hpi, the full blastocyst's trophectoderm (TE) began to herniate and hatch. By 114.3hpi a second internal blastocoel cavity formed appearing to divide the inner cell mass (ICM) within the *zona pellucida* (ZP). This resulting blastocyst proceeded to hatch as its discrete ICM migrated out of the ZP, along with its TE. TE cells from the original blastocyst then began to hatch at 117.5hpi at the same breached site in the ZP with its ICM visibly evacuating the ZP.

By 140hpi the blastocyst had split into two discrete blastocysts while hatching from the ZP. Both resulting blastocysts had clear and separate ICMs and TEs present. Biopsy of approximately 5 cells was performed for each TE, and the blastocysts were vitrified individually. Next Generation Sequencing (NGS) reported both blastocysts to be euploid.

Limitations, reasons for caution: This case may have been detectable without time-lapse imaging, as the splitting was completed prior to biopsy. More expert scrutiny of the images may result in earlier signs of twinning in progress being detected.

Wider implications of the findings: The nature of this detectable *in vitro* blastocyst splitting, indicates these embryos (if they implanted) to be monozygotic, dichoriol-diamniotic 'identical' twins. However – as single embryo transfer is the preferred treatment plan; they may be born years apart. These observations could shed light on the debated models of monozygotic twinning.

Trial registration number: not applicable

P-254 The outcome of artificial reproductive technologies (ART) cycles with transfer of frozen-thawed blastocysts depending on whether expanded on day 5 or day 6

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Study question: Is there a difference in ART cycle results after frozen embryo transfer (FET), depending on whether blastocysts were cryopreserved on day 5 or 6?

Summary answer: There's no statistical difference in the clinical pregnancy rate (CPR), life birth rate (LBR), miscarriage rate (MR) between embryos frozen on day 5 and 6.

What is known already: Currently, opinions differ regarding this topic. Previous studies demonstrated no difference in ongoing pregnancy rates between embryos frozen on day 5 (group A) or day 6 (group B) after FET. However, metanalysis (2019) suggested higher CPR and LBR after transferring embryos from group A rather than group B. It has also been established that ovarian stimulation leads to endometrial changes that result in deleterious effects on the implantation window and endometrial receptivity. Consequently, fresh transfers were excluded. Due to hormonal priming of endometrial receptivity, the same pregnancy outcomes should be expected with frozen-thawed blastocysts (day 5 vs 6).

Study design, size, duration: Retrospective cohort study was conducted between January 2015 and December 2018 with selected group of patients under 40 years of age. Group A consisted of 2275 cryotransfers of blastocyst expanded on day 5; group B included 170 cryotransfers of blastocyst on day 6. Both groups had an average of 1,52 embryos transferred per patient.

Participants/materials, setting, methods: Embryos were vitrified and warmed with Cryotop method (Kitazato, BioPharma). Blastocysts were scored according to Gardner and Schoolcraft grading system. Only expanded on day or 6 blastocysts of excellent and good (AA, AB, BA, BB) quality were selected. The embryos were cultured in CSC medium (Irvine Scientific) for 2-4 hours prior intrauterine transfer. The cycles with donor gametes, surrogacy and preimplantation genetic testing (PGT) were excluded. Statistical validity was assessed by Pearson's chi-squared test.

Main results and the role of chance: The rates of the CPR, the ongoing pregnancy rate (OPR) and the LBR between group A and B were 50,8% (1157/2275) vs 46,5% (79/170) (p=0,26), 37,4% (852/2275) vs 37,0% (63/170) (p=0,91), 36,5% (832/2275) vs 35,2% (60/170) (p=0,73) respectively and no significant differences were found in each category. Moreover, similarly there

were no significant differences in the miscarriage rate 26,0% (301/1157) and 21,5% (17/79) (p=0,37) as well
Limitations, reasons for caution: The study is limited due to uneven distribution of patients in both groups and by a low number of participants. The grading of blastocysts' quality is also subjected to a human factor.
Wider implications of the findings: This study confirms that frozen-thawed blastocysts do not seem to exhibit a difference in the CPR, OPR, LBR and MR whether they were expanded on day 5 or day 6. The cryopreservation of day 6 blastocyst can increase the chances of the patient for the positive outcome.
Trial registration number: not applicable

P-255 Hyaluronic acid-sperm selection significantly improves the clinical outcome of couples with previous ICSI cycles failure

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Study question: Does hyaluronic acid (HA) sperm selection improve the intracytoplasmic sperm injection outcome of couples with previous ICSI cycles failure?
Summary answer: In couples where previous first ICSI failed, selection of HA-bound spermatozoa significantly improved clinical outcomes respect to further standard ICSI.
What is known already: HA is the major component of the matrix surrounding the human oocyte and in physiological fertilization it plays an important role in sperm selection since only mature spermatozoa express specific binding protein and are able to bind to HA. Although several studies demonstrated better outcomes of ICSI with selection of mature HA-bound spermatozoa, such a beneficial effect of HA-ICSI is still controversial and to date no firm clinical guidance for the routine use of HA can be drawn. Further studies are needed to categorize patients that really might benefit from HA sperm selection before ICSI.
Study design, size, duration: A retrospective, longitudinal cohort study performed at a tertiary level public infertility center. We selected 164 couples who performed one or more failed ICSI cycles with low fertilization rate and poor embryo quality in the period 2010-2020 (n=164 cycles, group A), followed by other standard ICSI (n=99, group B) and/or HA-ICSI (n=96 group C) cycles. We included only fresh ejaculated sperm and fresh oocytes.
Participants/materials, setting, methods: Endpoints were fertilization, cleavage, top quality embryo, implantation (IR), clinical pregnancy (CPR), pregnancy loss (PLR), and live birth (LBR) rates. Comparisons among groups were performed using a generalized estimating equation model performed at patient level, to take into account the correlation between observations originating from the same woman. A p-value <0.05, after correction by female age at oocyte retrieval, was considered statistically significant.
Main results and the role of chance: The three groups were similar for number of retrieved, MII and injected oocytes. As regarding embryological outcomes, there was no difference in fertilization and cleavage rates between group A and C (fertilization: 47.55+29.88% *versus* 54.10+28.51%, p=0.096; cleavage: 96.19+12.70% *versus* 97.52+10.50%, p=0.519), nor between group B and C (fertilization: 60.30+30.73% *versus* 53.71+28.61%, p=0.112; cleavage: 92.26+20.540% *versus* 97.55+10.44%, p=0.106). Selection of HA-bound spermatozoa in ICSI significantly improved the embryo quality rate (63.78+35.55% *versus* 51.42+34.31% p=0.024) and the blastulation rate (43.44+25.55% *versus* 17.93+25.52%, p=0.001) respect to standard ICSI. Comparisons of clinical outcomes between group B and group C highlighted significant higher IR (26.16+40.47% *versus* 7.34+22.16%, p=0.0001), CPR/cycle (32.29% (31/96) *versus* 12.12% (12/99), p=0.0007, chi-square test), and lower PLR (12.90% (4/31) *versus* 41.67% (5/12), p=0.0398, chi-square test) in HA-ICSI respect to standard ICSI cycles. The LBR/cycle in group B was 10.10% (10/99) and in group C was 32.29% (31/96) (p=0.0029, chi-square test). No stillbirths as well as no malformations in newborns were recorded.
Limitations, reasons for caution: We are aware of the retrospective nature of the study performed in a single ART center.
Wider implications of the findings: This study identified couples with previous ICSI cycles failure as a category of infertile patients that really may benefit from HA sperm selection before ICSI.
Trial registration number: Not applicable

P-256 Time Lapse Imaging (TLI) acquired morphokinetic variables, nucleation errors and cleavage abnormalities are associated with live birth and may aid in de-selection of transfer embryos

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Study question: May the observation by TLI of morphokinetics, nucleation errors and cleavage abnormalities assist in de-selecting embryos before embryo transfer?
Summary answer: The combine predictive power of the association between the three biomarkers and live birth may aid in embryo de-selection
What is known already: Morphokinetic parameters and cleavage biomarkers are associated with treatment outcomes following *in vitro* fertilization (IVF). Nucleation errors observed by TLI have also been associated with IVF outcomes. It is also shown that nucleation error self-repair in pre-implantation embryos occurs, resulting in euploid blastocysts and live births. Biomarkers identified by TLI have been incorporated in developing algorithms to be used in selecting "the embryo" with the best potential for a live birth. However, the few randomized control studies (RCT) have not shown convincingly that TLI significantly improves live birth rate.
Study design, size, duration: Analyses of TLI data from transferred embryos, cultured in the EmbryoScope TM between June 2012 and August 2018, in a single IVF clinical setting were included. 2082 treatment cycles with Known Implantation Data (KID) for implantation and live birth were included in the analyses. Nucleation errors such as micronucleation, binucleation, and multinucleation were systematically annotated. Cleavage abnormalities such as direct cleavages, rapid and reverse cleavages were annotated for a minimum of 44 hours post insemination.
Participants/materials, setting, methods: Annotations for cleavage abnormalities, morphokinetic variables and nucleation errors, during a minimum of 44 hours, for 2959 transferred embryos were obtained from the EmbryoScope. The potential negative association between day 2 KID embryo biomarkers and implantation as well as live birth was assessed. The analyses controlled for potential confounding by adjusting for maternal age, infertility diagnosis, BMI, hormonal stimulation regime and insemination method.
Main results and the role of chance: Preliminary results were obtained regarding biomarkers in the form of nucleation errors, cleavage abnormalities and early embryo morphological attributes. Several of these biomarkers were significantly associated with implantation and live birth. Nucleation errors were associated with substantial decrease in implantation and live birth, but contrary to findings from other studies, none of the recorded nucleation error types precluded live birth. Many morphokinetically defined cleavage abnormalities were also shown to be significantly associated with implantation and live birth, with timings to 2-cells (t2) and second cell cycle (cc2) displaying the most prominent predictions for live birth probability.

Within each of the three biomarker groups, logistic regression models with implantation and live birth probability predictions displayed reasonable explanatory power regarding implantation and live birth. Combining all types of biomarkers lead to logistic regression models with substantially higher explanatory power than when the regression models only comprised a single biomarker group.

With a study of this size and *P* values for the basic findings predominantly being highly significant, the role of chance is likely to be limited. The statistical uncertainty may therefore be subordinated to the confounding caused by embryo transfer selection and further by exclusive use of embryos with known implantation
Limitations, reasons for caution: Only transferred embryos with KID data were analysed and hence the outcome of other embryos is unknown. Our study used mostly day 2 embryos, therefore generalisation up to blastocyst stage is

not possible. Our findings apply to our study cohort and may differ from findings in another clinical setting.
Wider implications of the findings: Our study provides knowledge about the role of TLI biomarkers and their potential for deselecting embryos for transfer. This will avoid transfer of lower quality embryos with lower chances of live birth. Incorporating such non-invasive de-selection strategies, alongside morphology may contribute to improving IVF outcome.
Trial registration number: NA

P-257 An unknown cause lead to polyspermy in IVF cycles and 0PN zygotes in ICSI cycles in male patient

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Study question: The patient sperm has normal morphology and motility, which paternal factors cause the abnormal fertilization in IVF/ICSI and what is the underlying mechanism?
Summary answer: A genetic mutation of BEX1 and decreased PLC-zeta has been found in patient, which may provide novel insights of polyspermy and pronucleus formation during fertilization.
What is known already: In mammals, pronucleus formation, a landmark event for fertilization, is critical for embryonic development. Abnormal fertilization refers to the abnormal number of pronucleus and polar bodies in zygotes during in vitro fertilization, with an incidence of 5-15%, among which the incidence of polyspermy and 0PN is about 2-10% and 30%. However, the mechanisms underlying pronucleus formation still unclear. More research has focused on oocyte activation, while paternal relevant abnormal fertilizations have been rarely established. The mechanism of how sperm and/or substances carried by sperm influence the physiological process of fertilization is also unclear.
Study design, size, duration: In our study, we first work on the preliminary observation and analysis of sperm morphology, structure and sperm chromosome number, and then made further analysis at the genetic level to find out the cause of this particular phenotype in this patient. We use of zone-free golden hamster ova test the fertilizing capacity and rescue the pronucleus formation with SrCl2.
Participants/materials, setting, methods: The patient, golden hamster, Papanicolaou stain, scanning electron microscope (SEM), Transmission Electron Microscope (TEM), Fluorescence in situ hybridization (FISH), Whole Exome Sequencing (WES), IVF, ICSI, Assisted Oocyte Activation (AOA).
Main results and the role of chance: During 2016-2018, they did 4 cycle assistant reproduction technology. Cycle1, conventional IVF(C-IVF), 9 MII oocytes, 9 3PN zygotes; Cycle2, ICSI, 10 MII oocytes, 10 0PN zygotes; Cycle3, donor-oocytes C-IVF, 6 MII oocytes, 6 3PN zygotes, and the donor did C-IVF get normal zygotes and embryos; Cycle4, donor-sperm C-IVF, 7 MII oocytes, 4 2PN zygotes, 3 useable embryos. Remarkably, clinical examination about male shows normal sperm semen parameters. Papanicolaou stain and SEM shows that the sperm of the patient has normal morphology. The TEM data shows that the spermatozoa with normal head morphology and intact 9+2 sperm flagella structure. In the sperm FISH analysis, Chromosome ploidy is haploid. We performed WES on the male, after exclusion of frequent variants and application of technical and biological filters, two homozygous missense mutations were identified in BEX1 (c.191G>A [p. W64X]), which has been few reports of male infertility. The western blot result show that the PLC-zeta was decreased in patient. After 10mM SrCl2 assisted oocyte activation, the zygote has the pronucleus formation in ICSI.
Limitations, reasons for caution: At present, we only observe sperm related factors (morphology, structure, chromosome number, genetic mutation). Next step is to detect the substances sperm carried (e.g. RNA-seq, proteomics). In this case, what is of great concern to us is the inconsistencies of the abnormal fertilization during the conventional IVF and ICSI cycles.
Wider implications of the findings: Many studies of fertilization mechanism, the main focus is on the maternal cytoplasmic factors, such as the Ca 2+ release initiate the fast block of oocytes. There are few reports about abnormal fertilization due to sperm factors. Our case may offer new insights for the study of fertilization.
Trial registration number: not applicable

P-258 Ubiquitin, in the human embryo secretome, is a biomarker for embryo viability: a potential predictor of live-births, post embryo transfer

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Study question: Whether embryo-secreted ubiquitin could serve as a predictive biomarker for embryo development and viability for assessing pregnancy outcome?
Summary answer: Embryo-secreted ubiquitin concentrations showed positive correlations with (a) developing embryonic stages, (b) implantation rates, (iii) live-birth rates. Their altered levels were associated with miscarriages.
What is known already: Human infertility affects 15-20 % couple and is mitigated by ART approaches. Poor biological-viability of in vitro developed embryos contributes to implantation failure and low birth rates(LBR). The current morphology-based embryo selection approach has shortcomings in identifying biologically-viable embryos capable of producing live-births. Earlier studies have identified ubiquitin as a biomarker for embryo developmental competence. However, there have been no studies on estimations of ubiquitin in embryo-spent medium samples (E-SMs) and their correlative analysis with embryo-quality score and pregnancy outcome. Hence, such studies are required to establish whether or not ubiquitin could be a biomarker predicting pregnancy outcome.
Study design, size, duration: This was a retrospective, multi-centric study performed between July 2018 and September 2020. A total of 574 E-SMs (from 574 individual embryos), from 325 infertile women, were analysed for ubiquitin levels. Frozen E-SMs post-thaw were subjected to sandwich ELISA (Mybiosource, USA). Correlation analysis was performed on ubiquitin levels with developing embryonic stages and their scores, implantation rates (IRs) and pregnancy outcomes in terms of LBR.
Participants/materials, setting, methods: We measured ubiquitin levels in E-SMs obtained from three embryonic stages *i.e.,* cleavage-stage (2-10-cells; n = 182), morulae (n = 102) and blastocysts (n = 290). Ubiquitin concentrations among three developmental stages were compared and analysed using the Student's *t-test*/ANOVA ($P \leq 0.05$), followed by *Tukey posthoc test*. Levels of ubiquitin were correlated (using Pearson/Spearman analysis) with (a) developing embryonic stages, (b) embryo morphology, (c) IRs, and (d) pregnancy outcomes in terms of LBR.
Main results and the role of chance: Of 574 E-SMs analysed, 540 (94.07%) had detectable ubiquitin levels (pg/ml) and they varied in an increasing manner across developing embryonic stages and, across the three clinics. We observed a significantly different ($p < 0.0001$) levels of ubiquitin in three sets of secretors *i.e* low (153.1 ± 5.4; n=219), medium (498.9 ± 15.7) & high (1615 ± 46.5) secretors. Levels of ubiquitin among three developmental stages were significantly ($p < 0.05$) different under FET, but not with fresh-ET categories. Ubiquitin levels were independent of cleavage-stage morphology score but showed a positive correlation with blastocyst grades. Also, we observed a significant ($p < 0.05$) positive correlation of ubiquitin levels with implantation rates. Importantly, ubiquitin levels were higher in E-SMs of embryos which gave live-births *vis-à-vis* those with no-births. Moreover, altered levels (very high low) were associated with those embryos which resulted in miscarriages. This is the first report which measured ubiquitin in individual hE-SMs from three developing embryos and

showed a development stage-wise positive correlations as well as a significant association ($p < 0.0001$) of ubiquitin levels with implantation and live-birth rates.
Limitations, reasons for caution: Observed variations in levels of ubiquitin across clinics could be attributed to (i) oocyte/sperm donors' variation and their infertility status (i) IVF-ET protocol differences. A large multi-centric cohort studies are required to establish the predictive value of ubiquitin for assessing embryo-viability and pregnancy outcome in term of live-births.
Wider implications of the findings: For the first time, our multi-centric study showed developmental stage-specific changes in ubiquitin levels. It could be a valuable biomarker of embryo-viability and to predict IR and live-births. Ubiquitin, as a biomarker, could be a valuable adjunct to currently practicing embryo score system for selecting transferable quality embryos.
Trial registration number: Not applicable

P-259 Blastocyst quality is associated with both the start and the duration of compaction after ICSI

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Study question: Is the start and the total duration of compaction related to embryo quality?
Summary answer: The timing of the start, the end and the total duration of compaction are associated with blastocyst quality grade in the IVF laboratory.
What is known already: Preimplantation embryo development follows a programmed timeline during which a series of critical events take place. One event typically occurring on day 3/4 post fertilisation is the formation of adherence junctions between blastomeres in a process called compaction. It is considered the first morphological event in the differentiation process of the mammalian embryo. Evaluation of developmental events are used to optimize the selection of the most competent embryos for transfer and/or cryopreservation in the IVF laboratory. It has already been shown that the time of full compaction is indicative for high-quality blastocysts with a higher implantation rate.
Study design, size, duration: A single-centre retrospective observational study including 74 ICSI cycles performed in 2020. Injected oocytes were cultured in blastocyst medium (Origio) in the EmbryoScope+ (Vitrolife) for 5/6 days. Embryos that reached the blastocyst stage were evaluated for the start of compaction, the time to reach full compaction and the total duration of compaction. These parameters were compared between good- and poor-quality blastocysts; the primary outcome parameter of the study was embryo quality.
Participants/materials, setting, methods: Only ICSI cycles with ejaculated fresh/frozen-thawed sperm and monitored in time-lapse incubator were included. All MNC, IVM and PGT cycles were excluded. Time zero was the start of ICSI. Good-quality embryos were full and expanded blastocysts with good-quality inner cell mass and trophectoderm (AA, AB, BA and BB according to Gardner and Schoolcraft (1999)). GraphPad Prism was used for statistical analysis. After testing for normality and homogeneity, unpaired t-test or Mann-Whitney test determined significant differences.
Main results and the role of chance: In this study, of the 528 included 2PN oocytes, 229 (43.4%) reached the blastocyst stage and 299 (56.6%) were arrested. Among the former, 131 (57.2%) blastocysts were classified in the good-quality group and 98 (42.8%) blastocysts in the poor-quality group. In general, human embryos compacted slowly while dividing further and the blastomeres moved during the compaction process. The start of compaction was heterogeneous (between 50.9 and 102.7 hours post ICSI; mean=80.0 hours), as well as the cell number at the initiation (between 4 and 18 blastomeres; mean=12 blastomeres). The time analysis showed that the embryos in the good-quality group started to compact significantly earlier than those in the poor-quality group (mean=78.6 vs 82.2 hours; $R^2=0.06$; $p<0.01$). We confirmed that blastocysts in the good-quality group reached full compaction earlier than those in the poor-quality group (mean=86.8 vs 93.8 hours; $R^2=0.17$; $p<0.01$). Furthermore, the total duration of compaction was significantly lower in the good-quality than in the poor-quality group (median=7.4 vs 10.7 hours; $p<0.01$).
Limitations, reasons for caution: As this is a retrospective study, the influence of uncontrolled variables cannot be excluded. The absence of the pregnancy outcome and live birth rate is a shortcoming and will be subject of a larger patient-to-patient study.
Wider implications of the findings: These results indicate that an earlier start and a shorter duration of compaction are associated with better blastocyst quality. These morphological events can be valuable additional parameters in selecting the embryo of better quality when using a time-lapse incubator.
Trial registration number: not applicable

P-260 Towards better explainable deep learning models for embryo selection in ART

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Study question: Can heatmaps generated by occlusion explain the patterns learned by deep learning (DL) models classifying the embryo viability in ART?
Summary answer: Occlusion experiments generate heatmaps that reveal which regions in frames of time-lapse video (TLV) are more discriminative for classification and prediction by the DL models.
What is known already: DL has widely been explored in ART for embryo selection. Depending upon input (video or image), different DL models classifying embryo viability are developed. However, whether the prediction is based on actual input features or random guessing is unknown. The embryo selection in ART is subjective. If the intention is using DL models' prediction to transfer, freeze or discard the embryo, explanations of how they interpret embryonic development features brings transparency and trust. In other areas, heatmaps are used for explaining DL predictions. The heatmaps can be a tool to understand patterns learned by DL models for embryo selection.
Study design, size, duration: We trained two separate DL models for predicting the presence of fetal heartbeat for the transferred embryos. We further used occlusion generated heatmaps to explain the predictions. For training, retrospective data was used. The input dataset consisted of 136 TLVs and corresponding patient data for 132 participants (128: single embryo transfers and 8: double embryo transfer) from both IVF and ICSI treatment. Each video was assessed by an embryologist.
Participants/materials, setting, methods: DL models (A as ResNet-18, B as VGG16) are trained for predicting the presence of fetal heartbeat on a single frame extracted from TLV after day three or later. Model A has a better recall (0.7) compared to B (0.5). Heatmaps explain the reason behind models' recall rate by visually representing patterns learned by them. Using occlusion filter size 30*30 with stride 14 and size 50*50 with stride 25, we generate heatmaps for both models.
Main results and the role of chance: The heatmaps generated using occlusion can represent visually the patterns discovered by the DL models when predicting the presence of a fetal heartbeat. Using occlusion filter size 30*30 with stride 14, we verified that Model B has lower recall because the heatmaps show that the model finds redundant features present outside the embryo region in many input frames. It could be interpreted that either the model has not learned relevant patterns or is more robust to noise. This representation of DL models equips us in better decision-making, whether to consider or discard the prediction or rather train the model further, preprocess training data or change network architecture. The heatmaps revealed that for frames where significant patterns learned by the models are within the embryo region, more weight was given to specific features like the inner cell mass, trophectoderm and some parts within the zona pellucida. Moreover, the heat maps generated using occlusion are independent of the underlying model's architecture as the same experiment settings were used for both models. For occlusion filter size 50*50 with stride 25, the expanse of input regions (in or outside the embryo) considered relevant could be visualized for both models A and B.

Limitations, reasons for caution: Heatmaps generated by occluding input regions give a visual representation of features in individual frames not directly on videos. Explaining DL models by heatmaps besides occlusion, other techniques (Grad-Cam) exist but were not evaluated. Furthermore, there is no quantitative measure for evaluating whether heatmaps are a good explanation or not.
Wider implications of the findings: The heatmaps make the patterns discovered by DL models visually recognized and bring forth the prominent portions of embryo regions. This will again improve understanding and trust in DL models' predictions. Visual representation of DL models using heatmaps enables interpreting a prediction, performing model analysis and determining scope for improvement.
Trial registration number: not applicable

P-261 The human embryo following biopsy on day5 vs day3: Implantation, cytoskeleton, ultrastructure and effects of endometrial damage/inflammation on receptivity as revealed by scanning electron microscopy

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Study question: Are there any differences in implantation, cytoskeleton and ultrastructure of embryos biopsied on day5 vs day3 and how endometrial damage/inflammation may affect receptivity and implantation?
Summary answer: No differences are observed in implantation rates but vitrification following day5 biopsy led to more cytoskeletal/ultrastructural anomalies. Infections and epithelial damage severely affected endometrial receptivity.
What is known already: Successful implantation is dependent on the correct synchronization of the window of implantantation with the transfer of chromosomally/genetically normal embryos, in a well prepared receptive endometrium. This is the first study to examine the effects of day5 vs day3 embryo biopsy by comparing implantation/pregnancy rates and by analysing cytoskeleton using Confocal Laser Scanning Microscopy (CLSM), and ultrastructure by Transmission Electron Microscopy (TEM). In addition, Scanning Electron Microscopy (SEM) was used on endometrial biopsies to assess possible uterine pathologies/inflammation that may be responsible for the failed implantation after PGT-A/M and if subsequent treatment can increase implantation/pregnancy rates in succeeding PGT-A/M cycles.
Study design, size, duration: 470 embryos were biopsied on day5 for PGT-A (n=152-37 cycles) or on day3 for PGT-A (n=162-29 cycles) and PGT-M (n=156-22 cycles). Following transfer of normal embryos, spare embryos, rejected for transfer following day5 or day3 biopsy were processed for Cytoskeletal analysis (n=30 fresh day3 biopsied, n=30 day5 biopsied/vitrified) or TEM (n=20 fresh day3 biopsied, n=20 day5 biopsied/vitrified). Also, patients with a –ve hCG test, underwent endometrial biopsy to detect infection/inflammation and assess receptivity.
Participants/materials, setting, methods: Cytoskeletal analysis was performed by embryo immunostaining with α-tubulin, γ-tubulin, acetylated-tubulin antibodies and DAPI or/ PI to visualise DNA. TEM analysis was carried out following embryo fixation in gluteraldehyde, incubation in osmium, aqueous uranyl acetate, dehydration through ethanol series, and immersion in Epon. Endometrial biopsies were fixed in gluteraldehyde solution and processed for SEM using standard methods. The study was conducted in an academic hospital with an IVF/PGD laboratory and 3 private IVF Units.
Main results and the role of chance: 162 embryos were biopsied on day 3 for PGT-A (29 cycles), 10 cycles had no normal embryos for transfer, 30 normal embryos were transferred in 19 cycles leading to 12/19 (63.2%) +vehCG/ET and 11/19 (57.9%) Ongoing pregnancy rate/ET. 156 embryos were biopsied on day 3 for PGT-M (22 cycles), 2 cycles had no normal embryos for transfer, 34 normal or carrier embryos were transferred in 20 cycles leading to 15/20 (75.0%) +vehCG/ET and 13/20 Ongoing pregnancy rate/ET (65.0%). 152 embryos were biopsied on day 5 for PGT-A (37 cycles), 8 cycles had no normal embryos for transfer, 34 normal embryos were transferred in 29 cycles leading to 18/29 (62.1%) +vehCG/ET and 16/29 (55.2%) Ongoing pregnancy rate /ET. Analysis of endometrial biopsies with SEM revealed bacterial infections, inflammation and epithelial damage. So far, 33.3% of patients who received intracavitary infusions-antibiotic treatment per os achieved a +vehCG/ET in their next PGT-A/M cycle. Cytoskeletal analysis showed that the majority of spindles examined in both day3 and day5 biopsied embryos were normal (85/114(74.6%) and 87/137(63.5). However vitrification following day5 biopsy led to more cytoskeletal/ultrastructural anomalies which included multipolar/abnormally shaped spindles, chromosome-bridging, chromosome-lagging and more vacuoles, lipofuscins. and distension of mitochondria.
Limitations, reasons for caution: Patients undergoing PGT-A have various aetiologies (heterogeneous group). The embryos used for cytoskeletal and ultrastructural analysis in this study were all diagnosed with either chromosomal abnormalities or single gene defects following PGT-A or PGT-M.
Wider implications of the findings: This is the first study to compare implantation/pregnancy rates, cytoskeleton and ultrastructure of day5 vs day3 biopsied embryos. The similarities observed in implantation/pregnancy rates, and the limited ultrastuctural and cytoskeletal anomalies identified confirm the procedures' safety and indicate in certain cases endometrial factors/inflammation responsible for failed implantation following PGT-A/M.
Trial registration number: not applicable

P-262 Comparison of embryo aneuploidy rate and reproductive outcomes of ART cycles using fresh and vitrified donor oocytes

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Study question: Is there any difference in blastocyst morphology, embryo aneuploidy rate and ART clinical outcomes when using fresh or vitrified donor oocytes?
Summary answer: Frequency of good quality blastocyst obtained from fresh oocytes is significantly higher compared to vitrified.No difference in embryo aneuploidy rates nor clinical outcomes were found.
What is known already: Oocytes vitrification is an efficient method that allows non only fertility preservation but also the creation of donor oocytes banks, optimizing clinical resources for patients undergoing Assisted Reproductive Technology. Although the benefits of donor oocytes vitrification are well known, some studies have shown that this cryopreservation process can induce spindle abnormalities and chromosomal changes, leading to aneuploidy. Comparative studies between fresh and vitrified oocytes to evaluate embryo developmental competence, aneuploidy and clinical pregnancy rate (CPR) are needed.
Study design, size, duration: This retrospective study includes ICSI cycles with fresh donor oocytes(N=2795) and vitrified donor oocytes (N=1225) between January 2019 and September 2020. Pre-implantation Genetic Testing for Aneuploidy (PGT-A) was performed on Day 5 and Day 6 blastocysts. Fertilization rate, blastocyst morphology, aneuploidy status and CPR were analysed and compared between the groups. Recipients were equally distributed in terms of maternal age (40.86 years) and previous history, sperm samples were also similar in profile and origin (fresh-frozen).
Participants/materials, setting, methods: A total of 266 subfertile couples participated in the study, ICSI was carried out in all cycles. Vitrification and warming protocols were performed with a commercial kit. All embryos were cultured to blastocyst stage in a Time-Lapse incubator and assessed by Gardner's blastocyst grading scale. PGT-A testing was performed on trophectoderm biopsies by Next Generation Sequencing (NGS). Single/double embryo transfers were performed in all cases. Odd-ratios were calculated,and Chi-square was performed for the statistical analysis.

Main results and the role of chance: A total of 266 patients underwent 289 donor oocyte cycles yielding an overall of 4557 oocytes. ICSI was performed on 2795 fresh and 1225 vitrified mature oocytes. Similar fertilization rates were achieved with fresh and vitrified oocytes (75.9% (2122/2795) and 75.2% (921/1225), respectively (P=0.6)) yielding a significant difference in blastocyst rate of 71.7% (1522/2122) and 62.5% (576/921) (OR 1,519; 95% CI 1,290-1,789; p<0.001). In addition, when blastocysts morphology was analysed, a significant difference was shown in the frequency of good quality embryos that decreases from 56.6% (861/1522) with fresh oocytes to 51% (294/576) with vitrified oocytes (OR 1,249; 95% CI 1,031-1,514; P<0.02). PGT-A testing of blastocysts revealed not significant differences in euploidy rates (73.6% in fresh oocytes vs 76.8% vitrified oocytes, P=0.2). With regards to clinical outcomes, similar results were found between the groups. A total of 322 embryo transfers were performed (237 from fresh and 85 vitrified) achieving a CPR of 48.9% (116/237) with fresh oocytes and 54% (46/85) with vitrified (P=0.7) and a pregnancy loss of 6.7%(16/237) in fresh oocytes and 11.7%(10/85) vitrified oocytes (P=0.1).

Limitations, reasons for caution: The study was conducted on a small number of cases. Further studies are needed to confirm our findings. Moreover, although the same stimulation protocol was used, donors from different background were included.

Wider implications of the findings: This study supports the use of vitrified oocytes in the laboratory routine without compromising clinical outcomes. Although oocyte vitrification may have an influence on embryo morphology, blastocyst rate, no impact of this cryopreservation process is seen on embryo aneuploidy, developmental competence and CPR.

Trial registration number: Not applicable

P-263 Life Whisperer™, an AI-based algorithm to select non invasively best quality blastocysts for transfer: A multicenter analysis

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Study question: Is the AI-based Life Whisperer™ (LW) tool, suitable to evaluate blastocysts quality and predict clinical pregnancy (CP) in couples undergoing ICSI cycles?

Summary answer: LW blastocyst score is comparable to the scores of other classification methods. This AI model showed high sensitivity and a comparable specificity for CP.

What is known already: The morphology grading is the most widely used method for the selection and classification of the embryos in clinical practice. However,this evaluation entails intervariability and intravariability decision among the embryologists.Recently, research has been focused on new embryo selection systems based on computer-assisted evaluation such as time-lapse with complex algorithms that allow the recognition of objective parameters of the embryo morphology.The implementation of these technologies requires substantial investments that are not available for all clinics.LW is a new embryo selection method based on AI,where specific hardware is not needed,as it is based on single blastocyst images taken with a routine microscope.

Study design, size, duration: Between 2017-2020, a total of 513 Day-5 blastocysts, after ICSI, comming from egg donation treatment were included in this retrospective-multicentre study.Day-5 embryos were evaluated with 3 classification methods:Gardner's blastocyst grade (GB), the computer derived-output Eeva (EV) and LW AI-supported system. The good quality blastocysts were first evaluated using the GB and EV scores and subsequently compared with the LW scores.The sensitivity and specificity of LW was assessed to validate this system as a clinical pregnancy predictor.

Participants/materials, setting, methods: A total of 513 Day-5 blastocysts, from 134 oocyte donation cycles, were evaluated first by GB score: expansion (1-6), inner cell mass and throphoectoderm (A-C).EV analyses the cell division timing P2 (2cells stage duration) and P3 (3cells stage duration) differentiating three categories:High,Medium and Low(VerMilyea et al.,2014).LW scores ranked 1-10 from a single Day-5 blastocyst HR Image performed on inverted microscope,with a threshold >5 for defining a viable blastocyst.T-test and ROC-curves were used for statistical analysis.

Main results and the role of chance: The average of LW score obtained from GB higher blastocyst expansion score (≥4) was 7.48±0.09, while the average of LW score obtained from GB lower blastocyst expansion score (<4) was 4.69±0.3 (P<0.001). The average of LW score yielded from GB good morphology of Inner Cell Mass and trophoectoderm (AA,AB,BA) was 7.98±0.1 while the average of LW score obtained from GB lower quality blastocyst score (BB,BC,CB,CA,AC) was 6.36±0.156 (P<0.001).The average of LW score resulted from EV High blastocysts was 7.42±0.17, while the average of this obtained from EV low score was 6.43±0.3 (P=0.009).A correlation between EV and LW score could be assesed, except for the blastocyst that are considered Medium score from EV.

Therefore, a strong correlation between GB and LW system, as well GB+EV and LW, was found and an equivalent usability of the LW tool could be confirmed.

The analyse of LW score for transferred embryos (N=156), using ROC curve, showed a high sensitivity (0,928) but a low specificity (0,154) with a threshold of 5. Regarding our data, ROC curve shows that a threshold of 8,46 could enhance the prediction of CPR because in this point the specifity value is higher than 0.5.

Limitations, reasons for caution: The LW score validation compared to GB and EV methodology was carried out on a small number of embryos. Additionally,not all embryos had been transferred at the time of the analysis. Thus to enhance the accuracy of these data and the specificity of the clinical prediction, a higher sample size is needed.

Wider implications of the findings: Blastocyst selection looks equivalent between all systems,but the LW tool is more objective and faster, saving time and costs significantly, without needing substantial hardware investments.

Additionally,the LW-system shows almost the highest sensibility and may also improve the specificity by self-learning feeding the AI-system, thus tailoring predictions to each laboratory unique environment.

Trial registration number: NA

P-264 Clinical relevance of re-expansion after blastocyst thawing

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Study question: Are there any differences in clinical outcomes after SET of re-expanded versus non-re-expanded blastocysts?

Summary answer: The transfer of re-expanded thawed blastocysts is associated with improved clinical outcomes.

What is known already: Improvements in embryo culture conditions, endometrial receptivity protocols and vitrification as a revolutionary cryopreservation technique have allowed the expansion of blastocyst stage transfers (Lieberman and Tucker, 2006; Stanger *et al*., 2012; Rienzi *et al*., 2017), increasing clinical pregnancy and implantation rates in IVF cycles.

The re-expansion of thawed blastocyst at the time of transfer has been considered as a good prognosis factor, but not always thawed embryos re-expand. To evaluate the relevance of this event, we compared the clinical results of the re-expanded embryos versus the collapsed ones after their thawing and transfer.

Study design, size, duration: A total number of 1.125 frozen-thawed blastocyst transfers were included in this retrospective observational study between January 2018 and December 2020. Seven hundred and eighty-six thawed blastocyst were fully expanded at the time of the transfer and 339 thawed blastocysts were non-re-expanded when they were transferred.

Participants/materials, setting, methods: 1.125 single frozen-thawed blastocyst embryo transfer (SET) cycles (802 from donated and 319 from autologous oocytes) were divided in two groups (re-expanded vs non-re-expanded). Positive beta human chorionic gonadotrophin (bHCG), pregnancy rate (PR), early miscarriage rate (EMR) and live birth rate (LBR) were compared between the two groups.

Blastocysts were thawed using an Irvine Scientific® Thaw kit, Irvine Scientific® and were transferring in culture medium (Global® Total® LP, CooperSurgical®).

Main results and the role of chance: During 2018, 190 re-expanded blastocyst and 94 non-re-expanded were transferred. Statistical significant differences were found in the percentage of positive bHCG (48.4% vs 30.9%, p<0,0048) and PR (39.5% vs 25.5%, p<0,0203), respectively.

In 2019, statistical differences were found in the LBR between 307 re-expanded blastocyst and 124 non-re-expanded (30.6% vs 12.9%; p<0,00001). Differences were also found in positive bHCG (50.2% vs 21.8%, p<0,00001) and PR (40.7% vs 15.3%, p<0,00001), respectively.

Finally, in 2020, 289 re-expanded blastocyst and 121 non-re-expanded were transferred, and significant differences were obtained in the percentage of positive bHCG (46.8% vs 22.3%, p<0,00001) and PR (32.9% vs 15.7%, p < 0,00001), respectively. Globally, all the variables analysed were statistically significant in favour of the re-expanded embryo group: positive bHCG (48.7% vs 24.5%; p<0,00001), PR (37.5% vs 18.3%; p<0,00001) and LBR (20.1% vs 9.5%; p<0,00001), except for EMR.

Limitations, reasons for caution: The inherent limitations to a retrospective design. Larger studies are warranted in order to reach robust conclusions on the subject.

Wider implications of the findings: Transfer of re-expand blastocyst could be a positive indicator of clinical outcomes. In case of non-re-expand embryos, transfer of two could be reasonable.

Trial registration number: NONE

P-265 Investigating the nanotoxicity of solid silica nanoparticles in gametes following in vitro exposure

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Study question: Do solid silica nanoparticles qualify as a new research tool for the *in vitro* transfer of compounds into gametes prior to Assisted Reproductive Technology (ART).

Summary answer: Solid silica nanoparticles (SSNPs) could be used as an intra-gamete delivery system to deliver therapeutic biomolecules into gametes prior to ART.

What is known already: Sperm-mediated gene transfer (SMGT) results in the production of transgenic embryos; however, the success rate of this technique is low. Nanoparticles are an efficient intra-cellular delivery system *in vitro*. Naturally cell-secreted nanoparticles are involved in the development of gametes. Mesoporous silica nanoparticles have been shown to carry large amounts of compounds and to interact with gametes without toxic effects, thus providing an alternative to naturally secreted nanoparticles. However, this technique is associated with some limitations, such as the size of these nanoparticles. SSNPs can be synthesised on a smaller nanoscale, thus providing higher potential to penetrate gametes and delivering biomolecules.

Study design, size, duration: This was an experimental *in vitro* study that investigated the effects of SSNPs on the motility of boar sperm and the degeneration of hamster oocytes, as determined by ooplasm shrinkage.

Participants/materials, setting, methods: SSNPs (20 nm) were conjugated with fluorescein diacetate-5-maleimide (FDA5M), a fluorescent protein. FDA5M-labelled SSNPS were incubated with boar sperm (N=3) at 10 and 30µg/ml/107 sperm for four-hours. Motility parameters were assessed by computer-assisted sperm analysis (CASA). Binding potential was evaluated by fluorescent microscopy. Hamster oocytes (7 oocytes/group) were incubated with FDA5M-labelled SSNPs at 100, 150, and 300µg/ml, for two-hours; ooplasm shrinkage was evaluated. Time/matched control sperm was incubated in phosphate-buffered saline and oocytes in KSOM.

Main results and the role of chance: Exposure to FDA5M-labelled SSNPs did not affect total or progressive sperm motility (P=0.6735 and 0.9606, respectively), average-path velocity or straight-line velocity after 4-hours of incubation (P=0.7459 and 0.8696, respectively) compared to controls. SSNPs at 10 µg/ml significantly increased sperm curvilinear velocity after 1-hour (P=0.0495) and linearity and straightness after 4-hours (P=0.0389 and 0.0312, respectively). SSNPs at 30 µg/ml significantly increased sperm linearity after 3- and 4-hours (P=0.0384 and 0.005, respectively). The proportion of sperm showing green fluorescence was significantly higher in the 30µg/ml dose of SSNPs than the 10µg/ml dose after 4-hours (P<0.00001). In oocytes, the zona pellucida remained morphologically intact and the ooplasm exhibited green fluorescence. The ooplasm of 42% of the oocytes at 300µg/ml showed ooplasm shrinkage (a sign of degeneration); no oocytes showed shrinkage at doses of 100 and 150µg/ml of SSNPs. The green fluorescence in the sperm head and the ooplasm indicated the ability of SSNPs to spontaneously interact non-invasively with these gametes either by surface association or by cell-internalisation. This could provide a safe and non-invasive intra-gamete delivery system for research purposes and clinical therapy. This system could be used to deliver specific agents into gametes prior to ART to improve outcomes.

Limitations, reasons for caution: The SSNPs are non-biodegradable; it remains unknown as to how gametes or embryos might react with SSNPs over long time periods. The nanotoxicity of SSNPs has not yet been investigated over the long term. SSNPs have still to be tested with embryos to evaluate their effect on embryonic development.

Wider implications of the findings: SSNPs could be functionalised to target the nucleus of mammalian gametes and embryos to act as a carrier for oligonucleotides and genes to correct chromosomal abnormalities and to provide genetic therapy in these gametes and embryos to treat hereditary diseases before intra-uterine transfer.

Trial registration number: not applicable

P-266 Morphometric assessment of Ratio of inner-cell-mass diameter to blastocyst diameter is an effective criterion to select best embryo for successful outcomes in single blastocyst transfer-cycles

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Study question: Can semi-quantitative measurement of ICM diameter vis-à-vis blastocyst diameter, calculated from photographic images, be a significant predictor of implantation potential in single blastocyst transfer cycles?

Summary answer: A simple, non-invasive measurement of ICM:Blastocyst diameter ratio is a potentially effective predictor of high developmental potential, implantation, live-birth rates in single blastocyst transfer cycles. What is known already: Correlationship between quantitative measurements of blastocyst morphology such as size and shape of ICM and its implantation potential were first reported by Richter et al. Widely used Gardner and Schoolcraft's qualitative scoring assessment is based on three major variables: expansion stage of blastocyst, cohesiveness of the inner cell mass (ICM), and consistency of trophectodermal (TE) cells. A top quality single blastocyst transfer yields implantation rate nearing 70% and live-birth rate about 50%. If an additional morphological but vital parameter evaluating ICM:Blastocyst diameter ratio is introduced to the Gardner's blastocyst gradation system, it may help enhance IVF success rates still further.

Study design, size, duration: A prospective observational cohort study of women (n=244) undergoing IVF treatment at our private fertility clinic from April 2018 until March 2020.

Women with their autologous fertilized oocytes undergoing extended culture and transfer of a single fresh, at least fully expanded (Grade 3) blastocyst with a measurable ICM diameter and blastocyst inner diameter on day 5/6, irrespective of age, cycle number and indication for treatment, were included. Cryopreservation cycles were excluded.

Participants/materials, setting, methods: Each blastocyst was evaluated for expansion grade, ICM and TE according to Gardner et al. conventional method. Additionally, ratio of ICM diameter w.r.t. blastocyst inner diameter was also calculated from the photographic images on screen using the Hamilton-Thorne software package embedded with their laser system. Measurements were done on blastocysts where expansion had occurred between 114 and 120 hours after insemination. Implantation rates and live-birth rates were the major end-points.

Main results and the role of chance: A total of 244 blastocyst transfers were performed in same number of women, and 130 clinical pregnancies were achieved (53.3%). The average age of the women was 32.5 ± 4.2 years. The blastocysts that implanted successfully had an average ICM:blastocyst diameter ratio of 0. 469±0.082), whereas blastocysts that did not implant had a significantly lower ratio of 0.325±0.09 (P <.0001). No statistical significant difference was found in the TE grade between the blastocysts that implanted successfully and those that did not. Out of the 130 pregnancies achieved, 89 (68%) resulted in the delivery of a healthy baby. After ROC analysis, a cutoff value for the ICM/blastocyst ratio showed equal rank for sensitivity (0.81) and specificity (0.72). The resultant positive predictive value was 78%, and the negative predictive

value was 74%. In our study, the conditional probability of achieving pregnancy upon transfer of a single blastocyst with an ICM:blastocyst ratio higher than the cutoff value of 0.4255 was 76%. This was significantly higher than the probability of pregnancy from blastocysts graded by conventional morphometry.
Limitations, reasons for caution: Our study is limited by the small sample size. Hence more multicentric studies are warranted to observe similar conclusion. Wider implications of the findings: The strengths of the study are the performance of single blastocyst transfers and using live births as the measurement endpoint. It is important to mention that embryo grading is inevitably subject to intra- and interobserver variations. However, with simple measurements of image data recording eliminates a degree of these variations.
Trial registration number: Not applicable

P-267 Characterising a novel embryo grading system for 4-cell embryos including symmetry, fragmentation, cell configuration, cell contacts per cell, distance between cells, and cell adhesion strength

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Study question: What other cell junction factors can be easily characterised from imagery of 4-cell embryos to assist in embryo classification and prediction of viability?
Summary answer: 4-cell embryo grading should not only account for symmetry and fragmentation, but also cell configuration and cell adhesion quality.
What is known already: 4-cell embryos are clinically classified according to cell number, symmetry and fragmentation without accounting for cell orientation or quality of cell junctions. Our previous work has focused on classification of 4-cell embryos according to overall embryo shape ("tetrahedral" versus "planar") using artificial intelligence. Our work, as confirmed by others, has demonstrated that embryo shape at the 4-cell stage is an important determinant of the ability of the embryo to reach blastocyst, utilisation, pregnancy and live birth. This is thought to be because of variations in intracellular communication between cells in embryos with different orientations, and consequently, different intracellular junction phenotypes.
Study design, size, duration: Using geometrical principles, possible permutations of 4-cell embryos (excluding redundant mirrored permutations) were identified and further classified based on shape and number of cell junctions. For ease of calculations, cells were assumed to be spherical, with at least one intracellular cell contact and symmetrical in size with other cells in the same embryo.
Participants/materials, setting, methods: The six distances between centroids of permutations for each configuration were calculated relative to the size of the cell, and the shortest distance between cell membranes. Adhesion was characterised from embryo imagery based on the overall shape of the cell, external angle between cells and the length of cell contact (the more spherical the cell, the larger the angle and the longer the cell contact point, the stronger the adhesion, adapted from Winklbauer,2015).
Main results and the role of chance: 4-cell embryos may be classified into 13 variant configurations: 1 typical Tetrahedral, 2 quasi-tetrahedral, 10 planar. These variants were classified according to number of cells with 0,1,2 and 3 intracellular contacts, leading to six possible configurations: 0004(tetrahedral), 0022(quasi tetrahedral/planar), 0040 (planar), 0121(quasi tetrahedral/planar), 0301(planar), 0220(planar). The number of total cell junctions in the embryo in each of these configurations was 12,10,8,8,6,6 respectively, with tetrahedral embryos (0004) having twice the cell contacts compared to planar embryos ($p<0.001$). Tetrahedral embryos have an advantage over the other embryo configurations in terms of better embryo communication, as demonstrated by the lower average and variation in distance and shorter sum of all intracellular distances between centroids (mean: 0.78 vs 0.94,0.98,1.04,1.09,1.19; stdev:0.06 vs 0.2,0.3,0.3,0.4,0.4,0.3; sum:4.7 vs 5.2,6.3,6.1,6.7,7.0,6.0 cell lengths) and between cells (mean: 0 vs 0.34,0.07,0.39,0.42,0.56; stdev: 0 vs 0.56,0.11,0.51,0.53,0.66; sum: 0 vs 2.71,0.43,3.13,3.38,4.46 cell lengths) observed in tetrahedral embryos versus other five configurations respectively ($p<0.001$).

Cell junctions were classified according to degree of cell adhesion: A:none (cells remain spherical in shape); B:weak (external angle between the cells is acute, there is a narrow visible cell junction); C:strong (external angle between the cells is obtuse with a wide visible cell junction). Limitations, reasons for caution: Follow-up studies will evaluate the impact of different cell shapes,cells without intracellular contact,and asymmetrical embryos. The proposed classification will be validated against a database of known outcome from 8 clinics from 6 countries to quantify the clinical implications of this classification,and the consistency of assessment by humans and AI .
Wider implications of the findings: It is clear that differences in intracellular communication between cells in embryos with different orientations, and different intracellular junction phenotypes is an important determinant of embryo viability. Our classification system allows for an easy to use and mathematically sound criteria for classifying 4-cell embryo cell junction quality.
Trial registration number: NA

P-268 Assessing the effect of media, oil and culture dishes on media osmolality and its dynamics in the culture system

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Study question: Can lab-related variables (media type, oil viscosity, micro-droplet volume and culture dish design) modulate media evaporation and improve its stability during culture?
Summary answer: Using dishes with pre-defined wells, big volume micro-droplets and high-viscosity mineral oil can help to reduce media evaporation and improve osmolality stability during embryo culture.
What is known already: Osmolality measures the number of solute particles present in a solution and is an important variable of a human embryo culture system. High ambient temperature and low humidity may induce evaporation in culture media, increasing its osmolality. In addition, recent tendencies in IVF laboratories, such as extending the embryo culture uninterruptedly until day 6/7 or the use of dry benchtop incubators, may intensify evaporation. Surpassing a 300mOsm/kg threshold can result deleterious for embryo development and impair clinical results. Different strategies (e.g. oil type/volume, dish type, micro-drop volume) have been proposed to reduce evaporation and stabilize osmolality during culture.
Study design, size, duration: Four variables were analyzed in their capacity to reduce media evaporation: type of culture medium, micro-droplet volume, oil viscosity and type of culture dish. Dishes were prepared with 5ml of oil and 50µl microdroplets (25µl were used for the comparison of micro-droplet volumes). Dishes were cultured in parallel in a dry benchtop incubator (AD-3100, Astec), and osmolality measured daily for seven days with a freezing point depression osmometer (Osmo1®, Advanced Instruments, accuracy ≤2mOsm/kg).
Participants/materials, setting, methods: The following comparison groups were analyzed: 1) Seven commercial single-step media with three differing initial osmolalities (approximately 260, 280 and >290mOsm/kg); 2) oil with high, medium or low viscosity; 3) 50 vs. 25µl microdroplets; 4) 35mm flat Petri dish vs. 35mm dish with defined wells. Temperature in the incubator was monitored continuously (T+Button, BrightSentinel), as well as room temperature and humidity (Octax Log&Guard, Vitrolife). All were stable at 37.3±0.05oC, 22.1±0.6 oC and 67.4±7.4%, respectively.
Main results and the role of chance: Evaporation occurred in all the studied groups, but its rate was modulated by various parameters. Culture dishes designed with pre-defined wells reduced evaporation when compared to regular Petri dishes (Increase 11.3mOsm/kg and increase 12.5mOsm/kg, respectively from day 0 to 7 ($P=0.007$)). Similarly, oil viscosity had an impact in osmolality stability during culture, with an increase of 14.7mOsm/kg, 16.3mOsm/kg and 19.2mOsm/kg observed when using mineral oil with high, medium and low viscosity, respectively ($P=0.009$). Finally, reducing the volume of the medium microdroplets from 50 to 25µl derived in higher evaporation rates, but without significant differences (Increase 14.7mOsm/kg and increase 15.8mOsm/kg, respectively ($P=0.325$)).

Different evaporation rates were observed between the seven studied culture media attending their three-differing initial osmolalities. Significant differences

were observed for a media respect another three media with differing initial osmolality (P= 0.001, P=0.01 and P=0.015). Their initial osmolality had a direct correlation with the maximum osmolality reached at the end of culture. Thus, media with a high initial osmolality (>290mOsm/kg) resulted in hyperosmotic media above the recommended 300mOsm/kg threshold by the end of culture and, by contrast, the studied media with lower initial values were able to maintain osmolality below 300mOsm/kg for the whole duration of the culture.

Limitations, reasons for caution: While a clear effect was observed by the studied variables, other parameters, such as oil volume or dish preparation techniques, could also play a role in osmolality maintenance and could be studied in the future. Additionally, these findings could vary between different centers and should be validated in each laboratory.

Wider implications of the findings: Osmolality has been shown to have a direct impact on embryo development, embryo quality and clinical outcomes. Carefully defining the consumables and methodologies used in the IVF laboratory will improve the stability of the culture system and, consequently, reduce the stress imparted to the embryos and gametes under culture.

Trial registration number: Not applicable

P-269 Effect of well-of-the-well culture as in vitro maturation system for human oocytes

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Study question: Can the Well-of-the-Well system (WOW), applied on denuded oocytes, improve germinal vesicle breakdown (GVBD) and maturation rate?

Summary answer: In vitro maturation (IVM) of denuded germinal vesicle (GV) oocyte using WOW culture system increases nuclear maturation competence when compared with droplet conventional culture

What is known already: Further research remains necessary to address the mechanism of oocyte maturation in order to refine culture conditions and improve the implantation rate of in vitro matured oocytes. Several studies on bovine oocytes have shown that oocyte-secreted factors (an uncharacterized mix of growth factors secreted by the oocyte) enhance oocyte developmental competence during in vitro maturation. These oocyte-secreted factors may accumulate at the bottom of the micro-well, as suggested for the WOW culture system. Previous reports suggested that diffusible factors secreted by individual oocytes probably accumulated in a micro-well WOW dish, may provide a suitable microenvironment for their in vitro maturation.

Study design, size, duration: A total of 879 GV collected between 2017 and 2019 were included in this study. They were randomly allocated into two experimental groups: (1) single-cultured oocytes (SC) that were cultured individually in micro-droplets, and (2) group-cultured oocytes (WOW) that were cultured in a microwell culture system using the WOW dish (culture dish for time lapse incubator). The nuclear maturation was assessed after 24 hours and 48 hours of IVM

Participants/materials, setting, methods: GV oocytes were obtained from 609 patients undergoing controlled ovarian stimulation cycles. Oocytes from the experimental group (1) were placed individually in conventional 25µl micro-droplets in a 35 mm dish. Oocytes from the experimental group (2) were placed in 80 µl droplet individually in each of 9 microwells of WOW dish. All GV oocytes were matured in a single step embryo culture medium, supplemented with human menopausal gonadotropin and synthetic serum substitute.

Main results and the role of chance: Mature oocyte (MII) was considered when we observed rupture of the GV and the presence of a first polar body in the perivitelline space during the first 24 or 48 hours of culture under inverted optical microscope. GVBD noted significant differences (p-valor = 0.000) between the study groups after culturing of 24 hours [GVBD: SC group; 70% (318/455) vs. WOW group; 83% (352/424)] and 48 hours [GVBD: SC group; 77% (319/416) vs. WOW group; 94% (398/424)]. The maturation rates (MR) showed significant differences (p-valor = 0.000) between the study groups after culturing of 24 hours [MR: SC group; 51% (233/455) vs. WOW group; 80% (338/424)] and 48 hours [MR: SC group; 71% (295/416) vs. WOW group; 91% (387/424)].

Limitations, reasons for caution: There is no data on cleavage and blastocyst rates.

There are no previous reports comparing the maturation rates in denuded human oocytes single-cultured in individually droplet or group-cultured in WOW dish.

Wider implications of the findings: Our results must be taken into account in order to improve the culture conditions for the optimization of the in vitro maturation technique in human oocytes from stimulated cycles.

We now provide evidence that group-cultured oocytes in WOW dish increase GVBD and maturation rates.

Trial registration number: Not applicable

P-270 Paternal age and reproductive potential with fresh and vitrified spermatozoa: An analysis of 11016 ICSI donor oocytes

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Study question: Does paternal age impair embryo aneuploidy and clinical outcomes in ICSI donor-oocytes cycles when fresh (FRs) and vitrified (VTs) spermatozoa are used?

Summary answer: Paternal age affects clinical outcomes, not embryo aneuploidy. With VTs, young and old men had similar outcomes. With FRs, young men had higher reproductive potential.

What is known already: Advanced paternal age is associated with low quality of sperm and an increase of reactive oxidative species, responsible of the DNA fragmentation, as well epigenetic disorders. In these men, the DNA repair mechanisms have a reduced ability to repair damaged DNA enhancing the likelihood of replication errors in the germ line. This genomic instability of the male gamete entails to chromosome abnormalities, generating potentially a negative effect on implantation and clinical outcomes. In addition, when the maternal oocyte repair mechanisms are not able to compensate quantitatively and qualitatively the sperm damages unrepaired embryos might develop.

Study design, size, duration: This retrospective study includes 848 couples undergoing 905 ICSI donor-cycles between January 2019 and February 2020, with similar quality and number of mature oocytes retrieved. Maternal age of recipients was 40.9±6 years and only those with previously failed cycles with their own oocytes were included. The clinical outcomes and aneuploidy were analyzed in two groups with the male partner being younger (M≤40) or older than 40 years (M>40).

Participants/materials, setting, methods: This study includes couples that underwent ICSI cycles with donor-oocytes using FRs and VTs ejaculates. Samples were analyzed according to WHO 2010 criteria. An in house-protocol (Vitri-Sperm®) was used to perform spermatozoa vitrification/warming. Embryo quality was assessed with time-lapse technology (Geri®), Aneuploidy Testing (PGT-A) was carried out on blastocyst's trophectoderm using NGS (Illumina®) and Fisher's Exact test was used for statistical analysis. P-value was considered statistically significant at a threshold of <0.05.

Main results and the role of chance: Fresh ejaculate was used in 192 cycles with M≤40 (concentration: 39.4±35 x 106/mL; motility: 35.1±16%) and 242 with M>40 (concentration: 36±34 x 106/mL; motility: 30±16%) yielding similar fertilization: 75.3% (1785/2369) Vs 75.6% (2232/2938). Comparing M≤40 with M>40 implantation decreased significantly from 66.6% (92/138) to 54.4% (79/145, P=0.03), Clinical Pregnancy Rate (CPR) from 68% (85/125) to 54.3% (75/138 P=0.03). Pregnancy loss from 15.2% (19/125) to 17.3% (24/138), not statistically significant.

Vitrified spermatozoa in 195 cycles with M≤40 (concentration: 4.9±7 x 106/mL; motility: 13.4±9%), and 276 with M>40 (concentration: 4.3±4 x 106/mL; motility: 13.9±12%) yielded significant difference in fertilization, 76.2% (1841/2416) Vs 72.4% (2386/3293, P<0.001), respectively. In M≤40 and M>40 implantation was 51.9% (40/77) Vs 49.1% (60/122) (p<0.05), CPR was 53% (38/71) Vs 54% (59/109). Pregnancy loss was 16.9% (12/71) Vs 13.7% (15/109), not statistically significance.

In M≤40 undergoing ICSI+PGT-A cycles (N=43) with FRs, euploidy was 71% (157/221) Vs 73.5% (256/348) in M>40 cycles (N=71). Implantation and CPR were equal in FRs groups, 77.1% (27/35) Vs 75.8% (44/58). Using VTs euploidy

in M≤40 cycles (N=63) was 71.9% (210/292), compared to 70.0% (279/399) in M>40 cycles (N=82). Implantation and CPR were higher in both groups, 76.5% (36/47) Vs 73.6% (53/72), not statistically significant.
Limitations, reasons for caution: Normo- and oligo-zoospermic patients, with quite different parameters and ethnicities have been included, allowing an analysis on a larger study population, but suitable of being analyzed further by subgroups. Implantation parameters like receptivity or immunologic disorders weren't addressed. No data have been included on perinatal and obstetrical outcomes for pregnancies.
Wider implications of the findings: Paternal age affects the clinical outcomes and embryo viability, it does not affect embryo aneuploidy when FRs and VTs are used. In ICSI donor-oocytes cycles with VTs, no significant difference in clinical outcome was found between young and older men. However, young men with fresh ejaculate have higher reproductive.
Trial registration number: N/A

P-271 Should intracytoplasmic sperm injection (ICSI) of delayed mature oocytes become a routine practice in the IVF Laboratory?

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Study question: Do delayed mature oocytes result in similar euploid blastocyst rates as their immediate mature sibling oocytes?
Summary answer: Once a blastocyst is obtained, delayed mature oocytes have similar euploid rates compared to immediate mature oocytes.
What is known already: Intracytoplasmic sperm injection (ICSI) of metaphase II oocytes few hours post oocyte retrieval is standard practice in IVF laboratories. Immature metaphase I (MI) and prophase I (GV) oocytes are usually discarded. Immature oocytes may mature overnight, after which ICSI can be performed. Studies demonstrated lower fertilization and blastulation rates for these delayed mature oocytes. However, live births have been reported from blastocysts transferred. The evidence available is not compelling, since most of the studies had either low sample size, no preimplantation genetic testing for aneuploidies (PGT-A), or the outcome was not compared to sibling MII oocytes at time of denudation.
Study design, size, duration: A single-center retrospective sibling oocyte study was performed between January 2019 and December 2020 at ART Fertility clinics Abu Dhabi, UAE. A total of 345 PGT-A cycles, with at least one delayed mature oocyte inseminated by ICSI, were included: 2506 immediate mature oocytes and 669 delayed mature oocytes.
Participants/materials, setting, methods: Following controlled ovarian stimulation, MII oocytes at the time of denudation were inseminated by ICSI/IVF (immediate mature). Immature oocytes (MI/GV) were cultured for 16-24 hours in fertilization medium and injected the next day if matured (delayed mature). Trophectoderm biopsy was performed on day 5/6/7 and samples were subjected to Next Generation Sequencing to screen the ploidy state of the blastocyst.
Main results and the role of chance: The 345 controlled ovarian stimulation cycles resulted in the insemination of 2506 MII oocytes on the day of oocyte retrieval (Day0) and 669 delayed mature oocytes on day 1. Normal fertilization rate was significantly higher in the immediate mature oocytes compared to delayed mature oocytes (68% vs 56%, p<0.0001). Similarly, the usable blastocyst rate was significantly higher in immediate mature oocytes (59% vs 19%, p<0.0001). On day 5 of development, a significantly higher-good quality blastocyst formation rate was obtained from immediate mature oocytes (65% vs 27%, p<0.0001). The rate of good quality blastocyst on the day of biopsy was significantly higher in the immediate mature oocytes group (76% vs 62%, p<0.015).

Fisher's Exact Test was performed to compare the euploid rate of blastocysts biopsied on day 5/6/7 originating from immediate mature oocytes or sibling delayed mature oocytes. The euploid potential of blastocyst biopsied showed no significant difference between the two groups (p=0.388).
Limitations, reasons for caution: The timing of MI/GV oocytes transition to MII stage was not recorded since the incubation was done in a benchtop incubator. Furthermore, the same sperm sample was used to inseminate immediate and delayed mature oocytes, which might contribute to the compromised embryo development due to increased sperm DNA fragmentation.
Wider implications of the findings: Insemination of delayed mature oocytes by ICSI, should be considered as a tool to increase patients' chances of obtaining a euploid embryo. Especially in cases where low yield of euploid embryos is expected.
Trial registration number: not applicable

P-272 The aneuploid embryo secretome

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Study question: Does the metabolomic analysis of the embryonic culture medium predict the embryo aneuploidy?
Summary answer: The presence and quantity of some metabolites in the culture medium can select euploid embryos for transfer.
What is known already: Advances in analytical techniques for metabolomics have brought the possibility of better tools for the characterization of molecules. Embryonic metabolism can be used as a good indicator of viability, regardless of the morphology of the blastocysts, since differences were observed in the metabolic activities between the days of embryo development and in the rates of live births.
Study design, size, duration: 17 patients had their embryos biopsied between January to July 2019 in a human reproduction laboratory. All cases had PGT-A indication and after the biopsy, the embryos were frozen. The culture medium samples were individually prepared for metabolites extraction according to the Bligh and Dyer protocol. Controlled ovarian stimulation and dose adjustments according to the response of each patient. The metabolomics analysis was performed by mass spectrometry.
Participants/materials, setting, methods: Ovum pick up will be performed 35 hours after r-hCG administration. The embryos were kept in individual 50ul drops until the blastocyst stage. The biopsy was performed in 26 blastocysts. The samples were sent to the 337 metabolites analysis by mass spectrometry. 15 molecules with the highest score on the PLS-Da was submitted the ROC curves to illustrate the power of the metabolic ploidy analysis. Besides, we performed the functional enrichment analysis for each group.
Main results and the role of chance: After the genetic analysis by PGT-a, 10 aneuploid embryos and 16 euploid embryos were found. Comparing the quantitative target metabolomic analysis of the 337 metabolites in the embryo culture medium, we observed the L-Alanine, Cytosine, Guanosine monophosphate, Homocysteine, Hypoxanthine, and Xanthine hiperrepresented in the aneuploid embryos, and the Citrulline, L-Glutamic acid, Kynurenine, L-Leucine, Methionine, Ornithine, L-Phenylalanine, L-Tyrosine, L-Valine were hiperrepresented in the euploid embryos. Through the ROC curve, we can verify AUC = 0.987. This result suggests that the analysis of euploid embryos through the metabolomic analysis of the culture medium is valid to be used as a noninvasive aneuploid diagnostic. The functional enrichment analysis shows the urea cycle and the glycine and serine metabolism as the principal function alter by aneuploid.
Limitations, reasons for caution: Small number of samples and not validate sample group.
Wider implications of the findings: Further studies are needed to validate these findings for the diagnostic of embryo euploidy.
Trial registration number: N/A

P-273 Effects of ovulation induction with GnRH Agonist (GnRHa) on oocyte and embryo quality at the mitochondrial level: A retrospective and experimental study

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Study question: Does the GnRHa trigger improve oocyte and embryo quality in patients younger than 40, and do mtUPPR have a role?
Summary answer: GnRHa trigger improves oocyte nuclear/cytoplasmic maturation, blastocyst utilization and downregulates HSP60 levels and upregulates ATF5 levels compared to hCG trigger. GnRHa trigger suppresses mitochondrial stress.
What is known already: hCG has been used for decades to achieve final oocyte maturation and, thereby, correct oocyte retrieval timing in connection with ovarian hyperstimulation protocols. As an alternative to hCG, a GnRH agonist has been used to trigger the endogenous release of LH (and FSH) in a fashion resembling the mid-cycle surge of gonadotrophins. GnRHa is as effective as hCG for the induction of ovulation. It has been very well known that the GnRHa trigger improves oocyte nuclear maturation, embryo quality, and implantation rate, but the underlying mechanism remains unknown.
Study design, size, duration: 3054 women younger than 40; oocytes retrieved more than 10 (up to 20) analyzed. Male infertility was excluded. Ovulation triggered either by hCG (n=1368) or GnRHa (1668). Female mice were divided into three groups as control, hCG-treated and GnRHa-treated group. Superovulation was performed by FSH + hCG or GnRHa. Oocytes were collected 13 hours after hCG/GnRHa injection. ATF5, BiP, and HSP60 levels were analyzed by Western blot. Statistical analysis was performed using Student's t-test.
Participants/materials, setting, methods: This study has two parts. i) RCT and ii) Experimental. In the experimental part, three months old female BALB/C mice (25-30 g) were used and divided into three groups (n = 20/group) as control, hCG-treated and GnRHa-treated group. Superovulation was performed by administering an injection of 5 IU FSH (i.p.) and hCG (i.p.) or GnRHa (20 mg/kg) i.m. Oocytes were collected 13 hours after hCG/GnRHa injection. ATF5, BiP, and HSP60 levels were analyzed by Western blot.
Main results and the role of chance: The mean age (34.8 vs. 35.2 years), total gonadotropin dose (2176 vs. 2230 IU), and the number of oocytes picked up (14.9 vs. 13.4) were not statistically different among GnRHa and hCG group, respectively. No LH rise or any OHSS was noticed in any groups.

Oocyte maturation (79.8% vs. 75.9%), oocyte diameter (as a marker of cytoplasmic maturity) (10198 µm2 and 9474 µm2), fertilization rate (78% vs. 72%), and embryo utilization rate (52% vs. 47.2%) were significantly higher in GnRHa group compared to hCG group, respectively.

HSP60 level (activated by mtUPR) was statistically higher in the hCG group compared to the GnRHa group (55% vs. 22%, $p<0.05$ respectively). On the other hand, the ATF5 level was significantly higher in the GnRHa group than the hCG group ($p<0.0001$).
Limitations, reasons for caution: The limitation is that this is a proof-of-concept study to reveal the mechanism of good embryo quality with GnRHa trigger.
Wider implications of the findings: This application offers convenience and simplifies the IVF protocol with a better oocyte and embryo quality while reducing Ovarian Hyperstimulation Syndrome (OHSS) risk during IVF care
Trial registration number: not applicable

P-274 Undetectable viral RNA in follicular fluid (FF), cumulus cells (CC) and endometrial tissue in SARS-CoV-2 positive patients

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Study question: Is there any indication for presence of viral RNA in FF, CC, immature oocytes or endometrial biopsy (EB) of SARS-CoV-2 patients undergoing ovarian stimulation?
Summary answer: Viral RNA is undetectable in FF, CC and EB with RT-PCR. However, S-protein expression on corona radiata cells suggests susceptibility to SARS-CoV-2 infection.
What is known already: The effects of a SARS-CoV-2 infection on the female reproductive system are still poorly understood. Theoretically, co-localisation of the angiotensin converting enzyme (ACE2) and transmembrane serine protease 2 (TMPRSS2) on human blastocysts implies susceptibility to viral infection, mediated by the coronavirus spike (S) protein. To date, SARS-CoV-2 RNA was undetectable in mature oocytes from COVID-19 patients, despite the expression of *ACE2* and *TMPRSS2*. The presence of viral RNA in endometrial tissue, immature oocytes, CC or FF has not yet been investigated in samples from patients with positive nasopharyngeal SARS-CoV-2 test.
Study design, size, duration: This is a prospective, single-centre, observational study including ten patients with a positive nasopharyngeal swab for SARS-CoV-2, performed 48 hours before oocyte retrieval (OR), from September 2020 to January 2021. A patient was eligible if she preferred to continue treatment following adequate counselling of the unknown but presumably low risk for vertical transmission. Since a freeze-all strategy was applied, an EB was performed.
Participants/materials, setting, methods: During OR, all protective measures were taken. Pooled FF, CC and EB from each patient were tested for viral RNA presence with RealStar® SARS-CoV-2 RT-PCR-Kit1.0 (Altona-Diagnostics). Ct values <40 were considered positive. EB was collected for pathological evaluation and cultured to obtain endometrial stromal cells (EnSC). Immature oocytes and EnSC were tested for S-protein expression by immunohistochemistry with anti-S antibody (MA5-35958, Thermo-Fisher Scientific) followed by Alexa Fluor™ 488-donkey-anti-mouse (Thermo-Fisher Scientific) and visualized with confocal microscopy.
Main results and the role of chance: SARS-CoV-2 RNA was undetectable in the pooled FF, CC and EB from all patients included in the study. Histological analysis of the EB showed no pathological modifications, including inflammatory reaction, as compared to biopsies collected from swab negative patients. After staining with anti-S antibody, cultured EnSC and immature oocytes tested negative for the S-protein. However, the binding of anti-S antibody was demonstrated on the corona radiata cells remaining on the zona pellucida after oocyte denudation for intra-cytoplasmatic sperm injection, indicating presence of SARS-CoV-2. In that case, the explanation for the undetectable viral RNA in CC could be that the viral RNA concentration remained under the detection limit of the currently used RT-PCR test.
Limitations, reasons for caution: This study was conducted in a small population (ten patients included) with different viral load, with mild or without symptoms of COVID-19. Another important limitation is the absence of validation of the RT-PCR protocol for the investigation of other types of samples than nasopharyngeal swabs.
Wider implications of the findings: The absence of SARS-CoV-2 RNA in all samples analysed represents a step further in reassuring a safe ART program for COVID-19 patients. However, the presence of S-protein on corona radiata cells warrants further investigation, since the theoretical possibility to infect human oocytes and/or embryos cannot be ruled out.
Trial registration number: NCT04425317

P-275 Development of a prediction model using machine learning on small noncoding RNA biomarkers for non-invasive selection of high-quality embryos for the in vitro fertilization process

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Study question: The aim of the study was to identify molecules in the embryo culture medium as important predictive biomarkers of high-quality embryos
Summary answer: The study identified 14 canonical iso-miRNA molecules that prognostically determine the quality of the embryo with a prediction accuracy with 95% sensitivity and 80% specificity.

What is known already: The quality of the embryo for the success of the IVF process is not specifically diagnosed, only morphological features (monitoring in the embryoscope) are considered. Embryo quality selection systems have likely reached their peak. The success rate of the IVF process is only 29%.; it is therefore necessary to look for other biomarkers. The oocyte itself can significantly predict the development of the early embryo,as it is a supplier of RNA and cellular mechanisms. However, collection follicular fluid is technically demanding. The probability of oocyte fertilization does not reach the required percentage therefore other embryological techniques multiply the economic costs.
Study design, size, duration: Women (n=734) who visited the IVF centre were recruited for the study. Oocytes were collected from 54 of them and used for IVF. After 4/5-day embryo cultivation, the best quality embryo was selected and used for implantation into the uterus. The culture medium has been collected from 60 embryos during 3 years (2018-2020). Written informed consent was obtained from all patients. The study has been approved by the Ethical committee of the Košice governing region
Participants/materials, setting, methods: We used fresh/frozen culture media of embryos selected using an embryoscope. Further, information regarding the success of IVF, pregnancy and IVF failure was collected. Culture media libraries of noncoding small RNAs (miRNAs) were examined using massively parallel sequencing on the Illumina platform. Obtained data was processed with freely available bioinformatic tools and machine learning. For methods with different models, the number of predictive biomarkers and specific prognostic-predictive molecules were selected.
Main results and the role of chance: The main results of the study specifically identify ncRNA molecules that prognostically and predictively select a high-quality embryo suitable for IVF transmission from a low-quality embryo with 95% sensitivity and 80% specificity with an average accuracy of 85% in 4 different models. We also determined the minimum of 14 miRNA as prediction biomarkers. The developed model can predict embryo quality from the culture medium based on ncRNA results from sequence data and set the cut-off value for the expression and significance of individual miRNA molecules with respect to embryo quality. Furthermore, positive and negative correlations of miRNA molecules with different distributions in a high-quality embryo compared to a low-quality embryo were determined. The molecules identified in the embryo culture medium were organized according to their importance, resp. significance based on their significance coefficient. So far, there is no evidence of pending patents regarding the distribution of specific canonical miRNAs and iso-miRNA molecules analysed by massively parallel sequencing in terms of biological competence and embryo quality determination with multifactorial consideration of its variation. This is the first study focused on the success of the IVF process based on embryo quality prediction.
Limitations, reasons for caution: Exploratory data need to be validated in a larger scale study.
Wider implications of the findings: The given miRNA molecules and the software model can be used as a safe,non-invasive diagnostic test for the selection of a highly competent embryo. Canonical and iso-miRNA molecules from the study can be used in other forms of diagnostic assays, such as specific embryo selection probes and, plate hybridization assay.
Trial registration number: non clinical trials

P-276 Factors associated with first oocyte retrievals affecting time to live birth – a retrospective study

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Study question: Which are the main factors on first oocyte retrievals influencing the time to achieve a live birth?
Summary answer: The number of oocytes collected on the first retrieval is the most important factor to decrease time to live birth.
What is known already: The goal of infertile couples when they attend a fertility clinic is to obtain a healthy baby as soon as possible. Cumulative live birth rate is today considered the most reliable estimate of ART success. However, the time used to achieve such goal is still not clear both to health professionals and patients. Although there is a general idea of which factors predict ART success, both clinicians and embryologists are still not aware of which variables can effectively influence the time to a live birth.
Study design, size, duration: We analyzed retrospectively 333 couples who performed their first fresh IVF/ICSI cycles from January 2015 to December 2018, along with their eventual subsequent FET and/or IVF/ICSI cycles, leading to 146 live births. The aim of this study was to use "Time" as an additional measure of ART success, and to identify which variable of the first oocyte retrieval has a major influence on time to live birth.
Participants/materials, setting, methods: We included in total 430 oocyte retrievals and 147 FET cycles. Oocyte donation cycles were excluded. Data were studied cumulatively until the childbirth (couples at risk) or until the last treatment record (censored couples). Cox Regression Model for survival analysis was used to study the variables that may influence the time to live birth, in order to take both confounding and collinearity into account.
Main results and the role of chance: We considered the date of the first oocyte retrieval as the starting point and the date of the first childbirth as the ending point to determine the time to live birth. The mean age of the woman at the first oocyte retrieval was 36.8±4.67, the cumulative pregnancy rate 43.5% (95%CI:41.77%;45.23%) and the live birth rate 34.0%(95%CI:32.32%;35.63%).

As variables for the Cox Regression Model we selected the woman's age and number of collected oocytes from the first oocyte retrieval. We also considered the number of frozen embryo transfers (FET).

We have found a positive association between the number of oocytes collected at the first oocyte retrieval and the period until obtaining a healthy singleton (HR=1.20;95%CI:1.105-1.297;p>0.001). Conversely, a negative association concerning the number of FET and time to live birth (HR=0.32;95%CI:0.18-0.562;p>0.001) was demonstrated. Importantly, woman's age, was not found to have a significant effect on time to live birth.

Our results indicated that the number of oocytes collected at the first cycle, but not woman's age, mostly affect time to live birth. Poor prognosis patients were found to be associated with several embryo transfers.
Limitations, reasons for caution: This is a retrospective study with a small number of cycles and freeze-all procedures were not included. Additional inclusion of freeze-all procedures in a larger study will be needed to confirm our preliminary results.
Wider implications of the findings: This study identified oocyte number at first collection as a major influence on time to live birth. Increased attention to the "Time" parameter will be helpful for health professionals and patients, to further personalize reproductive treatment procedures, potentially decreasing psychological burdens associated with ART treatments.
Trial registration number: not applicable

P-277 Time-lapse technology improves patient In-Vitro Fertilization experience

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Study question: Does patient access to time-lapse technology improve their cycle experience or is it simply perceived as another add-on?
Summary answer: Patients reported increased satisfaction and transparency in IVF treatment after viewing videos of their embryos growing.
What is known already: It is widely assumed that In-vitro Fertilization (IVF) treatments are costly and stressful for intended parents. During an IVF cycle, patients are often provided with minimal information regarding their embryo growth milestones, -and no static images of embryos for their personal retention. Time-lapse technology provides a method to visualize embryos continuously without disruption to the embryo's growth cycle. Many time-lapse studies have focused on the potential benefits associated with non-invasive embryo selection, but very few have entertained the idea about how this technology could be used to improve patient engagement and education pertaining to their cycle.
Study design, size, duration: An anonymous survey was conducted which included 192 patients over 8 months. This survey study focused on responses of patients after viewing time-lapse videos from *all* of their embryos in culture. Embryo video updates were provided to the patients on Day 3 and Day 7 of embryo culture. Patients received two surveys: One prior to egg retrieval and a second after receiving all of their embryo culture videos at the completion of their cycle.
Participants/materials, setting, methods: Patients at a private IVF clinic were able to self-elect enrollment in the survey-study at the start of their cycle.

Embryos were cultured in CSCM-NX (FujiFilm Irvine Scientific) in the Geri Time-Lapse incubator (Genea BioMedx) for up to 7 days. Development was monitored according to standard lab protocol and embryos were vitrified or biopsied accordingly. Patients were provided access to videos electronically with a brief summary and a follow-up phone call the following day.

Main results and the role of chance: 192 patients participated in the initial assessment survey at the start of their IVF cycle. 155 of these patients then partook in the survey at the completion of their cycle. 98% of patients completing the survey prior to their cycle felt that being able to view the videos of their growing embryos on Day 3 and Day 7 would add value to their IVF experience. Nearly all (97%) respondents felt that watching videos of their embryos in culture added transparency regarding embryo development and the laboratory environment. In the survey at the completion of the cycle, 96.8% (150) of participants reported that access to the videos added no additional stress to their IVF process. Very few (3.9%) reported that they were not happy with the quality of their videos. 142 (91.6%) participants reported that the embryo updates they received correlated with what they observed in their videos. Transparency in the laboratory through video viewing was reported by 93.5% of patients. In the feedback/comments portion, many noted that they were grateful for the experience and enjoyed participating in the study. Despite such high reviews of the technology and personal involvement, there was marginal interest in paying a premium for this service.

Limitations, reasons for caution: Patient responses were limited to a yes or no answers. Patients agreed to complete the two questionnaires, but they were not required to do so. Since there was no enforcement of survey completion, this could account for some of the discrepancy in study start.

Wider implications of the findings: Patients noted that they enjoyed the experience of watching their embryos develop near-real-time but did not feel it necessary for success. The majority stated that viewing the videos fostered their emotional attachment to their embryos. It is possible that the increased transparency leads to less anxiety and more patient trust.

Trial registration number: N/A

P-278 The embryo score provided by the KIDScoreD5 is correlated with implantation rate and clinical outcomes in fresh transfer

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Study question: Can the algorithm used by EmbryoScopePlus software predict implantation and clinical pregnancy in women of different age groups on fresh transfer?

Summary answer: The embryo score generated by KIDScoreD5 is highly related to the rates of implantation and clinical pregnancy in fresh transfers in women of different age.

What is known already: Artificial Intelligence algorithms use statistics to find patterns in large amounts of data and describe a non-biased approach to multiparameter analysis. Several algorithms have been described, but none has been adopted for universal use. KIDScoreD5 is the algorithm included in the EmbryoScopePlus system and classifies embryos according to the cleavage times and morphology of the blastocyst. Version 3, more current, includes the annotations of the number of pronuclei, the time of division for 2, 3, 4 and 5 cells, time to start of blastulation, and morphology of the Internal Cell Mass and trophectoderm.

Study design, size, duration: Retrospective study evaluated 86 embryos from January to December 2019 at the Reproferty clinic, grown at EmbryoScopePlus and transferred fresh on the fifth day of embryo development. The morphological and morphokinetic parameters were automatically evaluated by the software and in case of any mistake, they were manually corrected. The embryos were evaluated by KIDScoreD5 v3 in different scores from 0.0 to 9.9 and divided into 4 groups (0.0-2.5; 2.6-5.0; 5.1–7.5; 7.6 –9.9).

Participants/materials, setting, methods: The inclusion criterion was transfer of a single embryo with 1 gestational sac and positive FHB and transfer of two embryos with 2 gestational sac and positive FHB. Patients with progesterone on the trigger day ≥ 1.5ng/mL and/or with endometrium ≤7mm were excluded. The implantation and clinical pregnancy rates were calculated according to age group, G1: ≤35 years; G2: between 36 and 39 years old; G3: ≥40 years, within the embryo classification.

Main results and the role of chance: For patients in group 1 (n = 31 embryos), 33.4% of the embryos were classified between 2.6-5.0; 69.20% of embryos with scores between 5.1-7.5 and 57.10% of embryos with scores between 7.6-9.9, with 100% of embryos that implanted, regardless of classification, resulting in clinical pregnancy . For group 2 (n = 35 embryos), they only showed an implantation rate for embryos where the scores were 5.1-7.5 (33.4%) and 7.6 - 9.9 (71.4%) , with 100% being the clinical pregnancy rate in these groups. For patients in group 3 (n = 24 embryos), we also observed implantation only in groups of embryos with a score of 5.1-7.5 (37.5%) and 7.6-9.9 (18.5%) , but the clinical pregnancy rate was lower when compared to the other age groups of the patients, with 33.5% for embryos having a score between 5.1-7.5 and 50% for the group 7.6-9.9. Regarding the average score given by the classification of KIDScore Day 5 v. 3 for embryos that implanted, for patients aged 35 years or less, the average was 6.92; for patients between 36 and 39 years old, the average was 8.06 and for patients aged 40 years or older, the average was 7.32.

Limitations, reasons for caution: This project is limited because it is a retrospective study and evaluated embryos from a single breeding center. Multicenter and prospective studies are necessary to validate the universal use of the KIDScoreD5 v3 algorithm in time-lapse incubators.

Wider implications of the findings: The study showed the ability of KIDScoreD5 v3 to assist the embryologist in deciding which embryo to transfer fresh, according to the patient's age, in addition to the software being effective in automatic annotation of morphological and morphokinetic parameters. Validating an algorithm universally will improve embryonic selection.

Trial registration number: Not applicable

P-279 Effect of Ca2+ ionophore A23187 (calcymicin) on fertilization rates, embryo development and pregnancy in human assisted reproduction cycles

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Study question: Does Calcymicin improve reproductive outcomes of ICSI cycles in cases of fertilization failure and/or embryo blockage indications?

Summary answer: The application of the Calcymicin after ICSI improves reproductive outcomes, especially in cases with clinical indication of fertilization failure.

What is known already: According to the bibliography, deficiencies in the oocyte activation process frequently lead to failed ICSI cycles, and these can be corrected by increasing initial levels of calcium (Ca2+) in the oocyte using assisted oocyte activation techniques (AOA), such as the use of Ca2+ ionophores. Ca2+ Ionophores have been shown to trigger an initial Ca2+ spike in the ooplasm that activates Ca2+/Calmodulin dependent protein kinase II, which initiates the cascade of cellular events leading to oocyte activation. Previous results suggest that Ca2+ ionophore treatment can give live offspring after failed ICSI cycles.

Study design, size, duration: 270 oocytes collected from 17 patients who presented cycles with low fertilization rates and/or embryo blockage or poor quality embryos (according to ASEBIR's embryo classification criteria) were retrospectively analyzed. Oocytes were divided into two groups, a control group that underwent conventional IVF/ICSI and another group that underwent an ICSI cycle with AOA. Study groups were defined according to clinical indications and subgroups according to AOA or control. All data were collected from 2017 until 2020.

Participants/materials, setting, methods: Among the 270 oocytes of the study sample, 142 belonged to the control group and 128 belonged to the AOA group. The AOA group oocytes were activated for 15 minutes immediately after ICSI using a prepared solution containing the Ca2+ ionophore A23187, *CultActive©* (Gynemed, Germany). Fertilization rate and type, blastocyst formation rate, blastocyst quality, embryo kinetics, and pregnancy rates were analyzed, all of them were compared to FIV/ICSI cycles without oocyte activation (control group).

Main results and the role of chance: In the analyses of the whole sample of oocytes, the AOA treatment gave a fertilization rate of 72.5 %, which was significantly higher compared to 53.8 % of the control cycles ($p = 0.002$). Good quality blastocysts and pregnancy rates were also significantly higher than the control ($p = 0.01$). In the group with an indication of fertilization failure, a significantly higher fertilization rate was recorded compared to the control (65 % and 33 %, respectively). A higher rate of abnormal embryos with three pronuclei was also found compared to the control ($p < 0.001$). There were no significant differences in blastocyst formation rates, quality, or embryo kinetics ($p > 0.05$). In the group with an indication of embryo blockage/poor embryo quality, a significantly higher rate of good quality blastocysts and lower blastulation time were recorded compared to the control ($p < 0.05$).
Limitations, reasons for caution: The safety of the AOA technique with Ca2+ ionophore has not been fully demonstrated. In our study, none of the newborns had malformations, and gestational weeks and birth weights were normal. However, further studies on the safety of this technique are needed to implement it routinely in human reproduction clinics.
Wider implications of the findings: According to these findings, an increase in the initial levels of calcium in the oocyte through the application of the Ca2+ ionophore A23187 after ICSI improves the results of failed assisted reproduction cycles, especially in the case of those diagnosed with fertility failure, which is a clear indication for AOA.
Trial registration number: Not applicable

P-280 Changes in oolemma height during ICSI injection on day 0 is associated with day 5-6 blastocyst formation

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Study question: Does the oolemma response to ICSI injection on day 0 affect blastocyst formation on day 5-6 (d5/6)?
Summary answer: A large change in oolemma height during ICSI injection on day 0 is associated with lower blastocyst formation rates on d5/6.
What is known already: The oolemma changes in all dimensions (i.e. height, width and depth) and can exhibit different reactions in ICSI during needle injection. This is seen as instant rupture or with little needle pressure, normal rupture with the needle pushed approximately halfway through, or difficult rupture with repeated attempts or the needle passing 3/4 of the oocyte width. Previous studies have shown that these responses can affect degeneration and fertilisation rates on day 1, however, there is little research on its effect on blastocyst formation rates. Furthermore, most previous studies have used qualitative methods to assess oolemma response.
Study design, size, duration: This is a retrospective study using ICSI procedure videos conducted by four embryologists in a private clinic from 2013-2015. All videos of procedures which did not result in 2PN or in which the oocyte was not fully visible were excluded. Six operators categorised 455 videos (by majority vote) into four groups based on the oolemma response: oolemma breakage within 1/4, between 1/4 and 1/2, between 1/2 and 3/4 and beyond 3/4 of the oocyte's width.
Participants/materials, setting, methods: A U-Net neural network model was trained to extract the frame of maximum oolemma indent from each video which were validated by a human operator; any in which maximum indent occured after breaking of the oolemma were excluded. The ratio of starting to maximal indent width/height were calculated automatically and human-validated. Chi-squared tests were performed for each ratio vs d5/6 blastocyst formation. These results were compared with those obtained from purely human annotations.
Main results and the role of chance: From the purely human annotations, the percentages of oocytes in groups 1-4 respectively were: 3.3%, 85.3%, 11.4% and 0%. This variation in oolemma response may be due to the arrangement of thick and thin microfilaments or cortical granules in the cytoskeleton. When analysed with d5/6 blastocyst formation, these showed no significant result (p=0.12) which is consistent with findings using the model.

The artificial intelligence (AI) model processed 26 frames per second. During human validation of the ratios calculated at maximal indentation, 36% of width ratios and 31% of height ratios were rejected.

The proportion of blastocysts formed in the upper and lower quartile for each ratio was analysed. Both the upper (0.49) and lower (0.41) quartiles of the width ratios were not significant for d5/6 blastocyst formation. The lower (1.12) quartile of height ratios showed no significance, however there were significantly fewer blastocysts formed on d5/6 for the upper (1.18) quartile of height ratios ($p<0.025$). This subtle change in the height ratio, which was significant for d5/6 blastocyst formation was not taken into account when grouping oocytes any previous literature (and our human labelling).
Limitations, reasons for caution: This study was conducted at a single clinic so variations between clinics were not captured in the study and would need further collaborations to confirm the proportion of oocytes responses. Due to the small sample size, this study also did not identify any group 4 oocytes cultured until d5/6.
Wider implications of the findings: The grouping criteria in this study were more quantitative than previous work yet indicated no correlation between the oolemma group and d5/6 blastocyst formation. However, changes in the height which are hard to assess in real-time (and which have been neglected in previous literature) were seen to be significant.
Trial registration number: NA

P-281 Is it worth to go to blastocyst when there are less than 4 embryos on day 3?

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Study question: Are pregnancy rates similar with blastocyst transfer compared to D3 transfer for patient with a poor embryo yield.
Summary answer: In poor prognosis patients, more D3 embryos are needed to result in similar outcome compared to single blastocyst and it increases the multiple pregnancy risk.
What is known already: Good prognosis patients benefit from a blastocyst transfer rather than cleavage-stage embryo because day 3 morphology has limited predictive value for subsequent developmental. A Cochrane meta-analysis in 2016 found a higher live birth rate per transfer in the blastocyst group compared to cleavage-stage and no difference in miscarriage and multiple pregnancies.

However, in unselected patients, studies have yielded conflicting results and especially in poor prognosis patients at risk of transfer cancellation.

A threshold of four good embryos on the third day has been previously correlated with blastocyst yield and live birth rate compared with cleavage-stage embryo transfer.
Study design, size, duration: We analyzed the outcome of 1115 cycles with less than 4 embryos during 2019-2020 and compared the results between two groups of D3 and D5 transfers.
Participants/materials, setting, methods: Amongst 1115 study cycles, in 691 cycles a D3 transfer was performed and in 424 cycles a D5 transfer was performed. We compared transfer cancellation rates, mean number of transferred embryos and ongoing pregnancy rates between the two groups and also in subgroups with female age <37 and female age >37. The statistical analyses were done by Chi square and t-test for paired samples.
Main results and the role of chance: In the overall study population, the mean female age was 36.3 ± 4.3 years, the mean number of obtained embryos was 2.4 ± 1.0, the mean number of transferred embryos was 1.4 ± 0.8. 17.2% of the cycles resulted in transfer cancellation (6.2% in D3 transfer group and 35.0% in D5 transfer group). After D3 transfer the ongoing pregnancy rate (OPR) per transfer was 21.5% compared to 39.7% in D5 transfers ($p<0.05$). A similar pattern was observed in subgroups of age <37 years and >37 years with OPR per transfer significantly higher when D5 transfer was performed. Notably more embryos were transferred on D3 compared to D5 (mean number 1.4 for D3 and 1 for D5). Nonetheless, OPR were similar per cycle in both groups and subgroups of different ages.
Limitations, reasons for caution: A prospective randomized controlled trial is needed to confirm these results that are consistent with previously reports on retrospective and observational studies.

Wider implications of the findings: In poor prognosis patients with low embryo yield, D3 and D5 transfers result in similar OPR per cycle. Transferring at blastocyst stage is not inferior to D3, despite the high cancellation rate, and appears safer permitting a single embryo transfer to avoid multiple pregnancy.
Trial registration number: Not applicable

P-282 A time lapse analysis of 36,671 embryos to compare the incidence of early stage abnormal cleavage events in ICSI and IVF derived embryos

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Study question: Is this incidence of early stage abnormal cleavage events different between embryos created following ICSI compared with IVF?
Summary answer: Embryos derived from ICSI are more likely to exhibit abnormal cleavage compared with those from IVF. This difference is most marked in women ≥35 years.
What is known already: Time lapse imaging (TLI) has been instrumental in allowing detailed annotation of early embryo development to provide an objective aid for embryo selection in ART cycles. Amongst several abnormal cleavage events reported, rapid cleavage and multichotomous mitosis/direct cleavage, during the first days after fertilisation have been demonstrated to be associated with lower blastulation rates, reduced implantation potential, increased aneuploidy and poor pregnancy outcomes. With ICSI being utilised commonly, and being the insemination method of choice in some clinics, the incidence of abnormal cleavage was investigated in association with insemination method, ICSI or IVF.
Study design, size, duration: The incidence of abnormal cleavage events was evaluated in a large multicentre retrospective analysis of 36,671 embryos from 6689 patients treated in 8 IVF clinics enabled with time lapse imaging, between 2011 - 2019. This constituted 10931 IVF embryos and 25740 ICSI embryos.
Participants/materials, setting, methods: Following ICSI or after IVF fertilisation check, embryos were time-lapse imaged every 10 minutes and annotated using the EmbryoScope. Second cell cycle durations were calculated as follows: time to reach 3-cell (t3) from 2-cell (t2) (t3-t2 = cc2). These were analysed using a welch t-test as three groups of abnormal cleavage: direct cleavage/trichotomous mitosis (DC) - where cc2=0 hours(h), rapid cleavage within 2h (R2) - where 0<cc2<2h and rapid cleavage between 2-5h (R5) where 2h<cc2<5h.
Main results and the role of chance: The incidence of DC, R2 and R5 in the whole cohort of embryos was 5%, 8% and 9% respectively. In the subpopulation of IVF embryos the incidence of DC, R2 and R5 was 4%, 8% and 9% respectively. In the subpopulation of ICSI embryos the incidence of DC, R2 and R5 was 6%, 8% and 9% respectively. The incidence of DC was significantly higher in ICSI embryos compared with IVF ($p<0.001$) whilst R2 and R5 were the same. ICSI derived embryos had a mean (± SE) cc2 value of 9.39 ± 0.03h, compared with 9.56 ± 0.05h for IVF embryos ($p<0.0038$). Examination of data split by maternal age demonstrated that ICSI oocytes from women of advanced maternal age (≥35) also had significantly more embryos exhibiting rapid cleavages R2 and R5 than IVF oocytes ($p<0.007$). There were no significant differences however, in rates of abnormal cleavages between ICSI and IVF in embryos from women aged <30 ($p=0.06$).

Male-factor diagnoses showed no significant differences in abnormal cleavage values between ICSI or IVF in all three abnormal cleavage categories.
Limitations, reasons for caution: This analysis could not control for all potential confounders therefore it is possible that the increased abnormal cleavages observed in this investigation are a result of another, or combination of factors. Despite quality assurance programs being in place across all clinics, there is a risk of annotation bias.
Wider implications of the findings: There is a higher incidence of early abnormal cleavage in embryos derived from ICSI, particularly in those from women of increased age and this research may help elucidate the reasons for this and add to the debate regarding the appropriateness of the increasing use of ICSI.
Trial registration number: not applicable

P-283 Combination between amino acids profile of the spent culture media and morphokinetics parameters of human embryos to determine its viability

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Study question: How to determine human embryo viability noninvasively before embryo transfer?
Summary answer: We propose that the combination of the amino acid profile of an individual embryo with its morphokinetics will provide noninvasive tool to determine its viability.
What is known already: It was already known that human embryos at early cleavage require non-essential amino acids, while at the 8-cell to blastocyst stages, a mixture of non-essential and essential amino acids. Amino acids have important roles during embryo development. Acting as biosynthetic precursors,buffers of intracellular pH in the embryo, antioxidants, energy sources and regulators of metabolic function and signaling pathways. Many studies have used time-lapse to analyze human embryonic development including the process of fertilization and assessment of early events and introducednoninvasive prognostic markers which predict embryo development and correlate it to IVF treatment outcomes.
Study design, size, duration: This study was a prospective cohort study approved by the Clinical Trial Ethical Committee of Faculty of Medicine, Alexandria University according to ethical standards of scientific research (Serial number: 0303721).Thirty females aged 30.13 ± 4.83 years undergoing ICSI cycle in the Madina Fertility Center, during the period of March 2018 to November 2019.202 MII oocytes were incubated individually in embryoscope.
Participants/materials, setting, methods: Embryos (n=161) were divided on Day 5 into two groups –developed embryos "Group D" (embryos that developed to blastocyst) and arrested embryos "Group A" (embryos remain at cleavage stage and fail to develop to blastocys).Developed embryos (Group D) included 99 embryos, and Arrested embryos (Group A) included 62 embryos. For each group, morphokinetic developmental points using embryoscope and the different amino acids concentrations in spent culture medium were analyzed using LC- mass spectro etry.
Main results and the role of chance: On one hand, the first appearance of pronuclei (TPNa), t2, t4 and CC2 in group D occurred significantly earlier than those of Group A.Analysis of 19 essential and non-essential amino acids in spent culture medium of each embryo in the two studied groups D and A showed a significantly higher concentration of two essential amino acids L-Valine (145.73 ± 150.96) and L-Phenylalanine (61.59 ± 55.78) in Group D than their concentration in Group A (104.58 ± 33.58, 44.24 ± 14.61, respectively , $p \leq 0.05$).and significantly lower concentration of three non-essential amino acids L-Tyrosine (62.56 ± 41.03) , L-Cysteine (19.48 ± 11.90), and L-Alanine (136.0 ± 389.83) observed in Group D when compared to Group A (69.57 ± 20.78, 22.37 ± 8.59,145.33 ± 165.22, respectively, $p \leq 0.05$) .Group D had higher levels of L-proline than Group A, P=0.010*.
Limitations, reasons for caution: It is important to note, that results were developed on a data set from one clinic with different stimulation protocols, a

	Group D	Group A	P
TPNa	11.36 ± 2.93	12.82 ± 3.58	0.004*
t2	27.09 ± 3.26	33.65 ± 14.61	<0.001*
t3	37.01 ± 4.05	40.14 ± 13.95	0.274
t4	39.78 ± 4.75	43.64 ± 13.70	0.043*
t5	49.74 ± 6.01	50.37 ± 13.61	0.666
CC2	9.92 ± 3.50	5.19 ± 13.41	0.010*
S2	12.29 ± 6.10	8.58 ± 14.38	0.078

multicenter data and a correlation with the stimulation protocol used should be involved in future studies, in addition a larger sample size to avoid high standard deviation is recommended

Wider implications of the findings: We can conclude that amino acid turnover is independent of the traditional morphological assessment of embryos and it may reflect its viability. The prospective combined use of amino acids profile of individual embryo and its morphokinetic parameters may contribute to introduce a new noninvasivs tool that may improve implantation rate

Trial registration number: 0303721

POSTER VIEWING

ENDOMETRIOSIS, ENDOMETRIUM AND FALLOPIAN TUBE, AND BENIGN DISORDERS OF THE ENDOMETRIUM AND FALLOPIAN TUBE

P-284 Changes in protein expression due to metformin treatment and hyperinsulinemia in a human endometrial cancer cell line

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Study question: The aim of the study was to identify new target proteins/pathways that are affected by metformin treatment in endometrial cancer cells in a proteomic approach.

Summary answer: The expression of 1,300 different proteins were investigated, of which 80 proteins with the most prominent changes were presented and some discussed in detail.

What is known already: The incidence of endometrial cancer (EC) has increased over the past years. Metabolic diseases such as obesity, type II diabetes mellitus (T2DM), and associated conditions (i.e. polycystic ovary syndrome (PCOS), insulin resistance) lead to elevated levels of circulating estrogens, which promote EC development and progression. Metformin, an insulin-sensitizing biguanide drug, commonly used in the treatment of T2DM, especially in obese patients, displayed anti-cancer effects in various cancer types, including EC. Different proteins and pathways have been suggested as potential targets, but the underlying mechanism of action of metformin's anti-cancer activity is still not completely understood.

Study design, size, duration: In the present *in vitro* study, EC cells were cultured in 5.5 mmol/L glucose medium (supplemented with 10 nmol/L ß-estradiol (E2)) and treated with metformin (0.5 mmol/L), insulin (100 ng/mL), or remained untreated for 7 d. The expression of 1,300 different proteins was detected in cellular extracts in an affinity proteomic approach and compared between the treatment groups in order to identify potential target proteins and pathways that contribute to the anti-cancer effects of metformin.

Participants/materials, setting, methods: The study was carried out with the EC cell line HEC-1A that represents a postmenopausal model with low E2 sensitivity. Proteins were extracted, quantified with the BCA assay, and protein expression was analyzed using the scioDiscover antibody microarray. Differences in protein abundance between samples were presented as log2-fold changes (log2FC) with significance for samples that displayed $|log2FC| \geq 0.5$ and adjusted $p \leq 0.05$. Pathway analysis was carried out with the STRING and DAVID databases.

Main results and the role of chance: The data revealed that metformin and insulin targeted similar pathways in the present study and mostly acted on proteins related to proliferation, migration and tumor immune response. These pathways may be affected in a tumor-promoting as well as a tumor-suppressing way by either metformin treatment or insulin supplementation. Results for the 80 most affected proteins were presented and the consequences for the cells resulting from the detected expression changes were discussed in detail for several proteins. The presented data helps identify potential target proteins and pathways affected by metformin treatment in EC and allows for a better understanding of the mechanism of action of the biguanide drug's anti-cancer activity. However, further investigations are necessary to confirm the observations and conclusions drawn from the presented data after metformin administration, especially for proteins that were regulated in a favorable way, i.e. AKT3, CCND2, CD63, CD81, GFAP, IL5, IL17A, IRF4, PI3, and VTCN1. Further proteins might be of interest, where metformin counteracted unfavorable effects that have been induced by hyperinsulinemia.

Limitations, reasons for caution: The results were obtained from an *in vitro* study with human cancer cell lines, and thus cannot be easily extrapolated to patients.

Wider implications of the findings: In the context of a hyperinsulinemic environment, further proteins might be of interest, i.e. AMFR, CCND2, CD63, ERBB3, EZR, GFAP, IRF4, PI3, PLCG2, SORL1, VEGFA, VTCN1, SPP1, and TM9SF2. Here, a metformin-induced insulin-sensitization might be able to counteract unfavorable effects on protein expression profile that have been induced by hyperinsulinemia.

Trial registration number: not applicable

P-285 Controlling semi-invasive activity of human endometriotic stromal cells by inhibiting NF-kB signaling pathway using aloe-emodin or aspirin

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Study question: Does inhibiting nuclear factor-kB (NF-kB) signaling by aloe-emodin (AE) or aspirin (Asp), as anti-inflammatory compounds, suppress the invasive activity of stage IV human endometriotic stromal cells?

Summary answer: Eutopic endometriotic stromal cells (EuESCs) seem to have a semi-invasive activity which is largely suppressed by AE or Asp.

What is known already: Inflammation and its master regulator, NF-kB, have been implicated in the development of endometriosis. Inhibition of NF-kB pathway using small molecules ameliorated disease progression and reduced the lesion size; nevertheless, underlying mechanism is not fully understood.

Study design, size, duration: In this cross-sectional study, a total of 8 infertile patients with proven endometriosis and 8 women without endometriosis (Control group) undergoing infertility treatment cycles, were enrolled between October 2018 and December 2019. The invasiveness of collected endometriotic stromal cells before and after treatment with AE or Asp, was analyzed and compared with the control group.

Participants/materials, setting, methods: The eutopic endometriotic and healthy endometrial biopsies were digested and the single cells were cultured. Gene and protein expression of proliferation, adhesion, and invasion markers of eutopic endometriotic stromal cells (EuESCs) with and without treatment with AE or Asp, as well as control endometrial stromal cells (CESCs) were analyzed using q-PCR and immunofluorescence staining, respectively. Cell migration capacity was assessed by wound closure assay.

Main results and the role of chance: We observed an association between NF-kB overexpression and higher proliferation/adhesion capacity in EuESCs. TNF-α, as a known NF-kB inducer, further potentiated this association. EuESCs at stage IV, displayed silent invasive and migratory behaviors. Pretreatment of EuESCs with AE or Asp significantly attenuated NF-kB expression and reduced proliferative, adhesive, invasive and migratory activity.

Limitations, reasons for caution: Due to some adverse effects observed following treatment with AE or Asp on the normal activity of EuESCs, more investigations on possible toxicity of the treatment, must be considered.

Wider implications of the findings: We suggest that both Asp and AE (as potent NF-kB inhibitors) may be useful as a supplement to conventional endometriosis treatments.

Trial registration number: not applicable

P-286 Uterine vascularity in women with previous caesarean section and its potential role in implantation failure: a retrospective cohort study

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Study question: Does a previous Caesarean section affect uterine vascularisation the day of embryo transfer?

Summary answer: 3D vascularisation parameters show less uterine irrigation in patients with previous Caesarean section
What is known already: A recent retrospective cohort study demonstrates that previous Caesarean section impairs live birth rates after assisted reproductive treatment (ART) compared to a previous vaginal delivery. Furthermore, it has been hypothesized about the mechanisms by which post-cesarean section niche may diminish clinical pregnancy rates. One of the hypothetical process mentioned has been a distorted contractility of the uterus caused by fibrosis, which can influence in the vascularisation of the endometrium.
Study design, size, duration: We retrospectively studied the uterine contractility and 3D vascularisation parameters in women who had an embryo transfer at the Instituto Bernabeu of Alicante, between 2018 and 2020 with one recurrent implantation failure (at least two good quality blastocysts transferred from egg donation treatment).
Participants/materials, setting, methods: Patients with large myomas (more than 4 cm), adenomyosis or polyp were excluded. In total, 196 patients were assessed on the day of embryo transfer which 12 patients had a previous caesarean section. Uterine contractility was analyzed using 4D ultrasound after 6 minutes of video recording. Vascularisation index and vascularisation flow index were assessed after the endometrial volume definition.
Main results and the role of chance: Baseline characteristics of both groups were comparable. 3D vascularization parameters were significantly lower in women with a previous caesarean section. Vascularization Index (VI) reached 0,8% in caesarean section group (CS group) versus 2,3% (p=0,038) and vascularization flow index (VFI) was 0,2 in CS group versus 0,8 (p=0,038) Despite uterine peristalsis showed less contractility in those patients with previous caesarean section (0,8 contractions per minute versus 1,1 contractions per minute), non-statistical differences were demonstrated (p=0,154)
Limitations, reasons for caution: This study is limited by its retrospective design and the low number of cases.
Wider implications of the findings: The lower 3D vascularisation indexes support a post-Caesarean section vascular-related impaired perfusion as a hypothetical mechanism. Its correlation with a possible impairment in the embryo implantation after fertility treatments warrants further studies.
Trial registration number: Not applicable

P-287 Uterine adenomyosis does not affect perinatal outcomes in ART treatments

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Study question: Is adenomyosis associated with worse clinical and perinatal outcomes in ovum donation cycles?
Summary answer: Adenomyosis was associated with reduced live birth rate per embryo transfer but not with increased risk of miscarriage or worse perinatal outcomes than controls.
What is known already: The effect of adenomyosis on IVF/ICSI outcomes are controversial as studies addressing this issue are limited in number and heterogeneous. Conclusions withdrawn from previous works differ regarding the prospective or retrospective design of the study. Two different metanalysis conducted showed that adenomyosis reduced implantation and clinical pregnancy rate and increased miscarriage risk. However, current data regarding perinatal outcomes of assisted reproduction techniques cycles in patients diagnosed with uterine adenomyosis is scarce.
Study design, size, duration: A retrospective cohort study in which 3307 patients undergoing ovum donation cycles were included. Patients who underwent single embryo transfer (SET) between years 2018 and 2019 were included and divided into two groups: adenomyosis (n=179) and controls (n=3218).
Participants/materials, setting, methods: Inclusion criteria consisted of patients in an oocyte donation program who had fresh SET on day 5 blastocyst stage development. Patients diagnosed with miomas and/or severe endometriosis and those who had undergone previous uterine surgical interventions were excluded from the study. Cases consisted of patients with a history of either focal or diffuse adenomyosis diagnosed via transvaginal ultrasonography (TVUS).
Main results and the role of chance: Clinical pregnancy rate per embryo transfer was 82/179 (45.8%) in those women diagnosed with adenomyosis versus 1869/3218 (59.8%) in control group (OR=0.57 95% CI. 0.41-0.78, $p<0.001$). Miscarriage rate was similar in the two study groups and differences found were not statistically significant, being 15/82 (18.3%) for adenomyosis and 309/1869 (16.5%) for control group. A lower live birth rate per embryo transfer was observed in women diagnosed with adenomyosis versus control, being 68/179 (38%) and 1560/3128 (49.9%) respectively (OR=0.615 95% CI 0.44-0.85, $p=0.002$). There were no statistically significant differences between childbirth delivery methods (vaginal versus caesarean section). Furthermore, means of gestational age at the time of delivery, newborn size and weight and incidences of low birth weight, preterm birth and admission in neonate intensive care unit (NICU) did not differ between the two groups. In addition, IVF and perinatal outcomes were similar in patients with diffuse adenomyosis compared to focal adenomyosis.
Limitations, reasons for caution: This is an observational study and thus possible confounders cannot be completely excluded. Diagnostic of adenomyosis is complex and, despite imaging via TVUS is both sensitive and specific, different criteria may be combined in order to fully assess the diagnostic.
Wider implications of the findings: Published literature has described how adenomyosis negatively impacts clinical outcomes in ART cycles; however, data regarding perinatal results is scarce. This study is of interest as it provides a first insight for clinicians showing that adenomyosis affects clinical but not perinatal outcomes in ovum donation cycle.
Trial registration number: Not applicable

P-288 Changes in gene and protein expression in human endometrial cancer cell lines after low dose metformin treatment over time

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Study question: Does metformin treatment lead to temporal expression changes of specific genes and proteins in endometrial cancer cell lines?
Summary answer: The expression of three different genes and proteins was investigated, of which all displayed changes over time.
What is known already: Endometrial cancer (EC) is one of the most common malignancies among postmenopausal women. A long-term estrogen effect on the endometrium often seen in women with obesity or the polycystic ovary syndrome (PCOS), as well as type II diabetes mellitus (T2DM) are well-known risk factors for the development and progression of EC. Metformin is a biguanide used in the treatment of T2DM patients and off label in women with PCOS. Moreover, metformin displays anti-tumor and anti-proliferative effects in various cancer types, including EC. In that regards BCL2L11, CDH1 and CDKN1A play an important role in apoptotic pathways, proliferation and invasion processes.
Study design, size, duration: The EC cells were cultured in normal (5.5 mmol/L) or high (17 mmol/L) glucose medium supplemented with 10 nmol/L ß-estradiol. The cells were treated with low dose metformin (1.0 mmol/L) for 2, 6, 24, 48 and 168 h (7 d). In addition, EC cells were treated with a combination of metformin and insulin (100 ng/mL) or remained untreated. Five independent experiments were fulfilled and untreated cells served as controls.
Participants/materials, setting, methods: The study was accomplished using two different human EC cell lines. HEC-1A represents an estrogen-independent EC, whereas Ishikawa represents the more common, estrogen-dependent EC. Proteins were extracted, quantified with a BCA assay, and the protein expression of BCL2L11, CDH1 and CDKN1A was analyzed by western blots. Furthermore, total RNA was extracted, transcribed to cDNA and Taqman real-time PCR was carried out to measure the expression of the associated genes, using fold change (FC) as parameter.
Main results and the role of chance: The expression of the selected genes, analyzed by RT-PCR, changed in both cell lines over time as follows: After 6 h, metformin induced a decrease in the expression of *BCL2L11* (FC=0.7) and *CDH1* (FC=0.75), whereas the expression of *CDKN1A* slightly increased (FC=0.95-1.35). After 24 h, *BCL2L11* expression increased in normal glucose groups (FC=1.3, high glucose: FC=0.93) and *CDH1* expression decreased in combination with metformin and high glucose (FC=0.7, normal glucose: FC=1.1). *CDKN1A* expression was increased by metformin in both cell lines after 24 h (FC=1.2-1.8). After 48 h of metformin treatment, expression for all three genes was only slightly changed (FC=0.9-1.0). After 7 d it was observed that the combination

of high glucose and metformin (i.e. like obese T2DM patients) led to an increased expression of *BCL2L11*, *CDH1* and *CDKN1A* (FC=1.4-2.9) in the presence and absence of insulin, whereas metformin induced a decreased expression of *CDH1* and *CDKN1A* (FC=0.5-0.75) in normal glucose medium. BCL2L11, CDH1 and CDKN1A expression was investigated at the protein level as well.
Limitations, reasons for caution: The results cannot be directly transferred to metformin treatment of patients, since the study was carried out *in vitro*. Additionally, further studies including more timepoints would indicate a more precisely gene and protein expression over time.
Wider implications of the findings: This is the first *in vitro* study showing the temporal changes of BCL2L11, CDH1 and CDKN1A expression, genes related to tumorigenesis due to low dose metformin over time, suggesting differentially pathways in long term metformin treatment using physiologically achievable metformin levels.
Trial registration number: not applicable

P-289 Progesterone receptor is not downregulated in endometrial epithelial compartment during embryo receptivity phase in assisted reproductive cycles

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Study question: Is progesterone receptor (PGR) downregulation disrupted within endometrial epithelial compartment, during embryo receptivity phase in assisted reproductive technology (ART) cycles?
Summary answer: PGR is not downregulated in endometrial epithelial cells from ART cycles during embryo receptivity phase.
What is known already: Progesterone (P4) promotes the downregulation of its own progesterone receptor (PGR). During the mid-luteal phase, PGR is downregulated in endometrial epithelial cells (EEC), a critical process for embryo implantation. Embryos are unable to attach to the maternal surface when PGR expression is sustained in EEC. Non-physiologic ovarian steroid produced or employed in ART cycles may alter endometrial development compromising its receptivity. Scarce information is available whether PGR is downregulated in EEC from ARTs including ovarian stimulation for *in vitro* fertilization (IVF) cycles or hormonal endometrial preparation for frozen thawed embryo transfer (HEP-FET).
Study design, size, duration: Cross sectional study including endometrial samples from fertile women during natural cycle (FNC, n=23), from infertile women submitted to IVF (n=19) and from infertile women who underwent mock HEP-FET (n=35). Samples were obtained between 2018-2019. Sample size was calculated considering a power of 90%, alpha error=0.05, an expected PGR expression of 2 and 0.5 in ART and FNC groups, respectively, having a standard deviation=0.9. At least 9 patients would be necessary in each group.
Participants/materials, setting, methods: Endometrial samples were obtained during mid-luteal phase scheduled 7 days after ovulation in FNC, 5 days after oocyte retrieval in IVF without embryo transfer or 5 days after P4 supplementation in HEP-FET. Immunohistochemistry was employed to quantify PGR using histologic score (Hscore). PGR mRNA levels were determined by qRT-PCR from EEC dissected by laser capture microdissection. Anova test was used for comparing means of Hscore and mRNA among groups. Statistical significance was established as $P<0.05$.
Main results and the role of chance: No statistical differences were found in demographic characteristics including age, body mass index or endometrial thickness. The PGR expression was reduced in FNC compared to IVF and HP-FET endometria (0.6 ± 0.1, 1.9 ± 0.9 and 2.2 ± 0.9 respectively; $P<0.0001$). The PGR mRNA levels from ECC dissected by laser capture microdissection were higher in IVF and HP-ET cycles compared to FNC (10.6 ± 3.1, 13.6 ± 2.3 and 0.8 ± 0.1 respectively; $P<0.0001$) corroborating the elevated PGR Hscore in EEC from ART cycles.
Limitations, reasons for caution: This is a descriptive study reporting failure of PGR downregulation in endometria from ART cycles with vaginal P4 supplementation during the luteal-phase. Whether interference or resistance to P4 signal is the mechanism involved in the failure of PGR down regulation in ART cycles needs to be determined
Wider implications of the findings: PGR downregulation within EEC was shown in FNC. The retained PGR expression detected in most ART cycles may interfere with embryo implantation and might explain the restricted pregnancy success. Future studies might reveal whether PGR evaluation in EEC can predict embryo implantation.
Trial registration number: Not Aplicable

P-290 Time-course analysis of endometrial miR/isomiR expression dynamics during hCG-primed menstrual-cycle phase transitions

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Study question: What is the qualitative and quantitative profile of microRNAs (miR) and their sequence variants - isomiRs, and how it changes during the menstrual-cycle phase transitions?
Summary answer: Time-course analysis of endometrial miR/isomiR profiles has shown that menstrual-phase transitions cause widespread and complex changes in *miR* gene expression and processing.
What is known already: Embryo implantation depends on the receptivity of the endometrium during the window of implantation, when ovarian hormones and genetic factors coordinate the development of the uterine lining and prepare it for embryo implantation. The most important factors for successful implantation studied so far are the embryo itself, the histological dating of the endometrium and its molecular genetic characteristics, including miRs. With the rapid development of next-generation sequencing technologies, it has become clear that *miR* genes have the potential to produce not only miR but also variants (isomiRs) thereof, which can differ in sequence and length and can be functionally significant.
Study design, size, duration: miR/isomiR landscape was assessed by small RNA sequencing of endometrial biopsy samples at 4 time points of endometrial cycle covering the proliferative and secretory phases. Healthy, fertile, female volunteers took part in the study lasting one and a half years. For accurate phase dating, human chorionic gonadotropin (hCG) was administrated, and ultrasonic, histological and hormonal assessments were done at each time point. Statistically significant data of miR/isomiR identification and expression dynamics was considered for analysis.
Participants/materials, setting, methods: Participant choice criteria - at least one child born, problem-free pregnancies, no diseases or allergies; hCG application time determined according follicle and endometrium ultrasound scanning, and ovarian hormone levels; endometrial biopsies taken at hGC (before hormone application), hGC+2, hGC+7, hGC+9 time points; small RNAseq completed by Karolinska Institute, Sweden; miR/isomiR identified using local Galaxy instance with an in-built workflow and tools developed by our laboratory; differential expression and target prediction evaluated with DESeq2 and miRDB,resp.
Main results and the role of chance: Within the cohort of patients, across the four study time points, the small RNAseq data revealed numbers of miRs and isomiRs to be changed. The largest statistically significant changes in their expression were found at LH+9. The miR families that showed the largest number of members with altered expression were miR125a, miR30d, miR449c, miR92a/b and miR99a. The expression levels tended to decrease in the miR125a and miR92a families and to increase in the miR10a and miR449c families during the three studied time points of the cycle compared to the proliferative phase. Among those affected, the number of isomiRs, including templated and non-templated isomiRs, was much higher than that of miRs. For example, the ratio of the significantly altered miRs/templated isomiRs/non-templated isomiRs was 6/16/11 at LH+9. Templated isomiRs of hsa-miR-148a-3p, hsa-miR-30d-5p and hsa-miR-449c-5p were among the most upregulated, while several templated

and non-templated isomiRs of hsa-miR-125-5p were the most downregulated at LH+9. Of particular interest are those isomiRs, in which the seed site is shifted compared to the reference miRs and results in altered target transcripts. Target prediction of the most affected isomiR of hsa-miR-449c-5p identified new targets of target scores much higher than of the reference miR.

Limitations, reasons for caution: IsomiRs are a source of novel biomarkers for clinical diagnosis. An important next step is the validation of the *in-silico* predicted miRs/isomiRs and their target transcripts by RT-qPCR in larger number of individuals. Expression profiles should be associated with the dominant cell type in the endometrial biopsy preparation.

Wider implications of the findings: MiR/isomiR signatures, together with those of their target mRNAs, can be applied to distinguish the endometrial phases, especially the implantation window, as well as for diagnosing endometrial dysfunction. It is worth investigating the possibility of miRs/isomiRs being used as biomarkers not only in endometrial biopsy but also in liquid biopsy.

Trial registration number: The Bulgarian National Science Fund КП-06 Н31/2

P-291 Searching for a suitable serum progesterone range at triggering day to achieve optimal cumulative live birth rate in high responders – Which range is better?

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Study question: Is there a suitable range of serum progestereone level at triggering day to optimize the cumulative live birth rate (LBR) in high responders?

Summary answer: Fom the point of view of cLBR, the optimal P4 range for triggering is between 1.5 to 2.5 ng/ml generally and in the high responders.

What is known already: It is well established that premature progesterone rise (PPR) affect adversely the pregnancy outcome in fresh embryo transfer cycle. It is inferred that PPR alters synchrony between endometrium and the embryos. However, detailed study of the effect of PPR on efficiency of oocyte retrieval , embryo quality and the subsequent cumulative pregnancy outcome is still lacking. Hence we sort to analyze the effect of PPR on the final cumulative LBR in our program especially focused on high responders.

Study design, size, duration: ART Database in our center was retrospectively reviewed. Total 1523 cycles between 20160101 and 20191231 were recruited under the condition of GnRH antagonist cycle with duration of ovulation induction for more than 5 days and available serum P4 level data on triggering day for data analysis for the relationship between serum P4 value and final cumulative LBRs.

Participants/materials, setting, methods: Cycles with serum P4 level < 1.5 ng/ml were defined as without PPR (Group A: n=1383). Cycles with serum P4 level >1.5 were defined as with PPR: P4 between 1.5 and 2.5 as Group B (n=113), P4 > 2.5 as Group C (n=27). Those high responding cycles (n=404) were analyzed similarly and separately as Group A' (n=304), B' (n=81) and C'(n=19). The statistics were carried out by SPSS-PC ver. 22.0 with $p<0.05$ as statistical significance.

Main results and the role of chance: Group A had significantly lower number of oocytes (9.8+8.0) retrieved as compared to Group B (19.3+11.2) and Group C (18.2+9.9). However there were no differences in fertilization rate, good embryo rates and BC formation rates between groups. The cumulative LBR (cLBR) were significantly higher in Group B (65.1%) as compared to Group A (40.9%, $p<0.001$) and Group C (37.0%, $p=0.008$). For the high responding cycles, Group B' also had marginally significant higher cLBR (75.3%) as compared to group A'(63.8% ; $p=0.051$) *and Group C' (52.6%; p=0.050). Comparisons between Group A' and C revealed significantly less oocytes retrieved but significantly higher blastocyst formation rates in Group A' and the resultant cLBR were comparable between these two groups. Comparisons between Groups B' and C' revealed comparable oocytes retrieved but significant lower blastocyst formation rates and cLBRs in Group C'. The baseline of the first part analysis revealed higher age and lower AMH in Group A, but comparable age and AMH between groups B and C.The lower cLBR in group A could be due to selection bias. The second part (high responders) showed comparable baselines between three groups. However, the case numbers are too few in group C' which might also result in uncertainty.*

Limitations, reasons for caution: Although the data revealed interesting, significantly different results between groups, this is only a retrospective analysis from our ART patient series. Selection bias could not be precluded. Analysis restricted to high responders could have a more balanced population for comparisons. However, more cases are needed to affirm the findings.

Wider implications of the findings: We still do not know the tolerable ceiling of serum P4 at the triggering day in high responders if future FET already planned. Pushing P4 value too high not only could not increase mature oocyte yields and possibly may decrease the number of available good blastocysts for optimizing final cLBRs.

Trial registration number: not applicable

P-292 Transcriptome signature of receptive endometrium is not affected by the presence of mild adenomyosis

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Study question: Does the presence of mild adenomyosis, a common acquired uterine anomaly, affect the endometrial gene expression levels during window of receptivity?

Summary answer: Mild adenomyosis has no significant influence on gene expression signature in the window of implantation (WOI).

What is known already: The improvements in imaging techniques have led to frequent detection of adenomyosis in women undergoing investigations for infertility. Although the data are conflicting, some clinical studies have shown that the presence of adenomyosis may interfere with embryo implantation and lead to poor pregnancy outcomes. The knowledge of molecular background that would lead to the phenomenon of altered endometrial receptivity in women with adenomyosis is limited and mainly demonstrated by selected candidate genes. Next-generation sequencing platforms enable genome-wide transcriptomic profiling of desired tissue samples and present a powerful tool to identify differentially expressed genes (DEGs) between women with adenomyosis and controls.

Study design, size, duration: We designed a prospective case-control study comparing women with sonographic evidence of mild adenomyosis (n=10) and women with normal uteri seeking assisted reproduction due to male factor infertility as the control group (n=10). All eligible women underwent infertility treatment at the Department of Reproductive Medicine and Gynaecological Endocrinology, University Medical Centre Maribor, Slovenia between years 2018 and 2020. For the present study, they were scheduled for cycle monitoring by urinary luteinizing hormone (LH) tests.

Participants/materials, setting, methods: Each endometrial biopsy was obtained in the presumed window of implantation (WOI) on days LH+7 to LH+9 after LH surge (LH+0). Isolated total RNA was applied for mRNA + lncRNA sequencing (RNA-seq) by Illumina Novaseq 6000. An aliquot of RNA samples was used to verify the WOI by the endometrial receptivity test "beREADY" (CCHT, Estonia). Gene Ontology and Reactome pathway enrichment analyses were conducted in ClueGO bioinformatics tool to study biological role behind obtained DEGs.

Main results and the role of chance: The R program language and Bioconductor packages were used to align generated RNA-seq reads on the human reference genome assembly (hg19) and to calculate gene expression differences between study groups using normalized counts per million (CPM)>10 in at least 10 samples. A total 233 DEGs ($p< 0.05$) was identified of which 126 genes were up- and 107 were down-regulated in adenomyosis compared to the control group. However, there was no significantly DEG according to the adjusted p-value. According to the beREADY test, all 20 samples were in receptive phase, however two samples were early-receptive and five were late-receptive. In a sensitivity analysis, all border receptive samples were removed and RNA-seq data sets were re-analysed only by 8 adenomyosis cases and 5 controls. A total of 382 DEGs ($p< 0.05$) were detected in adenomyosis group (216

up- and 166 down-regulated genes), again with no statistical difference between both groups after adjustment. Functional enrichment analyses of 233 and 382 DEGs identified pathways (adjusted p-value< 0.05) associated with *positive regulation of exosomal secretion* and *expression of IFN-induced genes*, respectively. The comparison of 233 and 382 DEGs revealed 28 common genes that may present stronger candidate of adenomyosis-related markers associated with endometrial receptivity.

Limitations, reasons for caution: Only mild adenomyosis was considered in this study, which is most commonly detected in women. The results could differ in women in severe cases of adenomyosis. Multicellular whole-tissue endometrial samples that were used for RNA isolation could mask gene expression differences of specific cell types between study groups.

Wider implications of the findings: According to our results of transcriptome analysis, the presence of mild adenomyosis has no significant influence on the gene expression signature during endometrial receptivity in natural menstrual cycle. Women being investigated for infertility can be reassured that this common acquired anomaly does not significantly influence the chances of successful conception.

Trial registration number: 0120-259/2018/16

P-293 Ovarian endometriomas are heterogenous for the steroidogenic function and the expression of estrogen and progesterone receptors

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Study question: Do all ovarian endometriomas have steroidogenic function and express estrogen and progesterone receptors?

Summary answer: No, they are heterogenous for the steroidogenic function and the expression of the estrogen and progesterone receptors.

What is known already: Excessive ectopic estrogen production and up-regulation of estrogen receptor-β, which drives inflammation together with aberrant progesterone signaling leading to impaired decidualization and establishment of ectopic endometrial implants together with down-regulated progesterone receptor (PR) expression are the cardinal molecular features of the disease. However, several fundamental questions still remain to be answered as to whether all ovarian endometriomas carry these molecular aberrations and are steroidogenically active; and if so, the amount of sex steroids they produce correlate with the level of expression of steroidogenic enzymes. We aimed to address these questions in the current study.

Study design, size, duration: A molecular research study on the surgical specimens collected between April 2020 and December 2020. Seven histopathologically confirmed benign endometriotic cyst capsules obtained from the patients undergoing laparoscopic excision of unilateral ovarian endometriomas without deep infiltrating endometriosis during early follicular phase were used in the study.

Participants/materials, setting, methods: The mean age±SD (range) of the patients were 32.8±4.9 (30-39). The mean endometrioma size was 5±1.2cm (5-7.5 cm). The samples were cut into equal size pieces of 0.5x0.5cm size and cultured for one day to measure their E2and P4production; and analyzed for the expression of steroidogenic enzymes with quantitative immunoblotting and for the expression of FSH-R, ER and PR with real-time qRT-PCR methods. Luteinized granulosa cells and ovarian cortex were set as references.

Main results and the role of chance: StAR expression was consistently observed in all samples. However, we noticed significant discrepancies among the samples regarding their steroidogenic function and the expression of aromatase and 3β-HSD enzymes. E2 production exhibited significant variation (from 5 to 1177pg/mL) from sample to sample despite comparable levels of aromatase expression. ER-β up-regulation as a cardinal molecular feature of endometriosis, was observed in all but one samples (1.46 to 5.48 folds, p<0.0001). However, its expression level did not correlate with either aromatase expression or the amount of E2 the samples produced. A similar phenomenon was observed in P4 arm of steroidogenesis. Even though 3β-HSD was expressed by all but one samples detectable amount of P4 was produced only by two samples (up to 15ng/mL). PR expression was down-regulated only in two samples (0.3 to 0.07 folds, p<0.0001), and significantly up-regulated in the other samples (1.2 to 4.7 folds, p<0.001). No correlation was found among the samples regarding the expression of PR, 3β-HSD and P4 output. FSH-R was detected in all samples at the levels comparable to ovarian cortex but its expression level did not show any correlation with ER, aromatase expression and E2 production.

Limitations, reasons for caution: These results need to be confirmed in studies with larger sample size and different types of endometriotic lesions.

Wider implications of the findings: The regulation of steroidogenic activity of endometriomas cannot simply be explained by the expression level of the steroidogenic enzymes, underscoring the importance of other mechanisms that post-translationally regulate their enzymatic activity and metabolism of estrogen and progesterone. PR is not always down-regulated and FSH-R is commonly up-regulated in ovarian endometriomas.

Trial registration number: not applicable

P-294 Mapping COVID-19 affected genes from blood in a Window of implantation co-expression network reveals a potentially compromised landscape

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Study question: Could the transcriptomic and functional landscape of the window of implantation be compromised by SARS-COV-2 infection?

Summary answer: Some of the main genes and pathways involved in the window of implantation are affected in blood of COVID-19 patients and receptivity could be affected.

What is known already: There is a concern whether SARS-COV-2 can disrupt assisted reproduction treatments (ARTs) and fertility in short and long terms. In the endometrium, it was found that genes related to the viral infection (ACE2, TMPRSS2/4, CTSL/B) are involved in menstrual cycle progression, especially in the Window of Implantation (WOI). However, there are no studies describing the transcriptome changes after the infection, and the changes that could affect receptivity and embryo implantation. Currently transcriptomic datasets are publicly available regarding virus infection effects in blood. The aim of this study was to integrate these blood effects with the gene expression during the WOI.

Study design, size, duration: A public dataset with blood transcriptome of 231 female COVID-19 patients and 30 female controls was downloaded from GEO. Meanwhile, 5 transcriptomic endometrial datasets in the WOI with patients without endometrial pathologies were also retrieved (n=44). Gene expression correlations (potential activations and inhibitions) were calculated in endometrium and filtered by blood differentially expressed genes for predicting the potential effects of COVID-19 in endometrial factor. Additionally, we discovered new endometrial genes involved in the infection repercussions.

Participants/materials, setting, methods: A gene co-expression network was built in Cytoscape with the WOI dataset [Pearson correlation = 0.65, only significant correlations; Power fit law R2 >= 0.8]. Differential expression was done for COVID-19 patients versus controls with limma and significant genes in blood were highlighted in the endometrial WOI network. Topological parameters were calculated by CytoHubba and network modules and related functions were analysed performing a Functional enrichment (BINGO). Statistical significance cut off was stablished in FDR<0.05.

Main results and the role of chance: After filtering by blood affected genes, 2051 genes were found differentially expressed in COVID-19 females in blood and mapped in the co-expression WOI network. Nine modules were highlighted being enriched in translational elongation, intracellular protein transport, endosome organization, vitamin D receptor binding, actin cytoskeleton organization, RNA splicing, among others. Important hubs in the endometrium that correlated with TMPRSS4 were: COBL, a gene that promotes formation of cell ruffles which are important or embryo adhesion (FC = -3.99, degree = 209); PKP2 (FC = -1.5, degree = 188) which could play a role in junctional plaques and knockdown in mice was reported to inhibit implantation; SOCS3, linked to unexplained

infertility and pregnancy loss, (FC -4.3, degree = 177); GPX3 involved in detoxification and usually highly upregulated during WOI was downregulated (FC -3.7, degree = 173). GPX3 also correlated with CTSB. TPRC/CD45, related to unexplained pregnancy loss and concentration of NK cells, was an upregulated gene (FC = 5, degree = 161) that correlated with CTSL. Upregulated genes with main connections in the network were: SERPING1 (FC = 5), which regulates complement activation and embryo-maternal immune modulation and SMARCAD1 (FC 1.5), involved in DNA repair and heterochromatin organization.

Limitations, reasons for caution: This is an in-silico descriptive study where differentially expressed genes in blood samples of COVID-19 patients were analysed in an endometrial co-expression network context. Studying a COVID-19 infected endometrium during WOI would help to confirm the results of this study.

Wider implications of the findings: Although ACE2 has been reported as not highly expressed during the WOI, this study describes potential genes and functions very important for embryo implantation affected after SARS-COV-2 infection. These findings evidenced how SARS-COV-2 could impact the efficacy of ARTs and should be taken into consideration for further research and implications.

Trial registration number: not applicable

P-295 Does endometriosis affect oocyte quality? An analysis of 13 627 donor oocyte recipient and autologous IVF cycles

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Study question: Does endometriosis affect live birth following donor oocyte recipient versus autologous in vitro fertilisation (IVF) cycle.

Summary answer: There was no significant difference in the live birth rate (LBR) in women with endometriosis undergoing donor oocyte recipient versus autologous IVF cycle.

What is known already: For infertile women with endometriosis, IVF is often considered as a treatment option. Lower implantation and pregnancy rates have been observed following IVF in women with endometriosis when compared to tubal factor infertility. It has been debated that lower pregnancy rates following IVF in endometriosis is due to both oocyte quality and the endometrium. To delineate whether endometriosis affects oocyte quality or the endometrium, we planned a study using donor oocyte recipient model where the recipient were women with endometriosis. We compared the LBR after oocyte recipient cycle with autologous IVF in women with endometriosis

Study design, size, duration: We obtained anonymised dataset of all the IVF cycles performed in the UK since 19991 from the Human Fertilization and Embryology Authority (HFEA). Data from 1996 to 2016 comprising a total of 13 627 donor oocyte recipient and autologous IVF cycles with endometriosis and no other cause of infertility were analysed.

Participants/materials, setting, methods: Data on all women with endometriosis undergoing fresh or frozen IVF treatment cycles were analysed to compare the LBR between donor oocyte recipient and autologous treatment cycles. Logistic regression analysis was performed adjusting for number of previous IVF cycles, previous live birth, period of treatment, day of embryo transfer, number of embryo transferred, fresh and frozen cycle.

Main results and the role of chance: There was no significant difference in the LBR in women with endometriosis undergoing donor oocyte recipient fresh cycles compared to women undergoing fresh autologous IVF cycles (31.6% vs. 31.0%; odds ratio, OR 1.03, 99% CI 0.79 – 1.35). After adjusting for confounders listed above, there was no significant difference in LBR in women undergoing donor oocyte recipient fresh cycles versus fresh autologous ART cycles (aOR 1.06, 99% CI 0.79 – 1.42).

There was no significant difference in the LBR in women with endometriosis undergoing frozen donor oocyte recipient cycles compared to women undergoing autologous frozen embryo transfer cycles (19.6% vs. 24.0%; OR 0.77, 99% CI 0.47 - 1.25). After adjusting for potential confounders, there was no significant difference in the LBR in women undergoing frozen donor oocyte recipient cycles compared with autologous frozen embryo transfer cycles (aOR 0.84, 99% CI 0.50 - 1.41).

Limitations, reasons for caution: Although the analysis was adjusted for several potential confounders, there was no information on classification of endometriosis to allow adjustment.

Wider implications of the findings: The current study design does not indicate endometriosis has an impact on oocyte quality given that the outcomes in donor oocyte recipient cycles are comparable with autologous IVF cycles. These findings need to be further studied and validated.

Trial registration number: Not applicable

P-296 Examining the link between environmental toxin exposure and uterine leiomyoma: a systematic review

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Study question: Is there an association between exposure to certain environmental toxins and the prevalence of uterine leiomyoma in women?

Summary answer: Some evidence was obtained to suggest an association between phthalate esters, bisphenol A, heavy metals, persistent organic pollutants and the prevalence of uterine fibroids.

What is known already: Environmental toxins are naturally occurring, or human made chemicals that can act as endocrine disrupting chemicals (EDCs) by binding and activating estrogen receptors in the body. Uterine fibroids, often called leiomyoma are non-cancerous growths occurring in the uterus. Though often asymptomatic, they can cause pain, infertility, pregnancy complications and are a leading cause for hysterectomy. The aetiology of leiomyoma is not fully understood but both estrogen and progesterone have been implicated in their growth. We aimed to investigate the epidemiological evidence for the association between EDCs and the prevalence of fibroids.

Study design, size, duration: We undertook a systematic review and in keeping with PRISMA guidelines, a structured search of Medline, Embase, Scopus, and Web of Science was conducted (to October 2020). Case-control, cross-sectional, cohort and experimental studies were included.

Participants/materials, setting, methods: The included studies analyzed the association between one or more toxins and the occurrence, or growth of leiomyoma in humans, including human cell lines. The types of toxins, patient characteristics, association and outcome, body concentration of toxin and confounding variables were extracted and analyzed. Quality assessment was performed using the Newcastle-Ottawa Scale.

Main results and the role of chance: In total, 34 studies were included. The majority (76%) of studies revealed a significant association between the exposure studied and the prevalence of uterine leiomyoma. In examining body burden in cases vs controls, phthalate esters showed an association with increased odds of uterine leiomyoma, except in one case where a negative association was observed. In vitro experimental studies examining the effect of alkyl-phenols such as bisphenol A (BPA), octylphenol (OP) and nonylphenol (NP) demonstrated that these environmental estrogens can act to promote the proliferation of leiomyoma cells through a number of mechanisms, typically including the estrogen receptor alpha (ERa) signalling pathway. There were conflicting results for the association between alkyl-phenols and fibroids in case-control studies. A positive association between cadmium was demonstrated in only two studies. There were conflicting results for the association between lead, mercury, arsenic and uterine fibroids. Several metabolites of organophosphate esters, alternative plasticizers, and persistent organic pollutants were associated with an increased risk of uterine fibroids.

Limitations, reasons for caution: Separating these exposures from the multiple other factors that could affect the outcome of leiomyoma is challenging, but an important issue for future research.

Wider implications of the findings: The link between some environmental toxins and uterine fibroids discussed is in agreement with previous literature. However, our review provides a more in depth analysis on specific dosage effects, odds ratios, and potential gene mechanisms of the exposures. This information could contribute to more accurate preventative measures.

Trial registration number: not applicable

P-297 Endometrium receptivity test in patients with failed embryo implantation

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Study question: Studying the receptivity of the endometrium for improving the synchronicity between the embryo and the condition of the endometrium.
Summary answer: Determination of the level of endometrial receptivity with PGT may significantly increase the effectiveness of IVF in patients with recurrent implantation failure
What is known already: Modern approach to the treatment of patients with recurrent implantation failure, using assisted reproductive technologies imply preimplantation genetic testing (PGT) of embryos for chromosomal abnormalities. However, in some cases, even with the transfer of a genetically complete embryo, pregnancy does not occur. A possible reason is a violation of embryo implantation due to a shift in the period of the "implantation window" . The ERA (Endometrium Receptivity Assay) is a personalized molecular genetic test specifically designed to analyze the level of endometrium receptivity for determine the period of the "implantation window".
Study design, size, duration: ERA test is carried out for patients with idiopathic infertility, in cases where repeated transfer of a genetically complete embryo does not lead to implantation and pregnancy: at least three attempts in the case of women under 37 years old and two attempts - for older women (without pathological morphological changes in the endometrium). The test was performed in a cycle with hormone replacement therapy (HRT) or a natural cycle.
Participants/materials, setting, methods: Were investigated the endometrial Pipell biopsy specimen in a cycle of hormone replacement therapy (HRT) or a natural cycle. Endometrial examination was implemented using the Endometrium Receptivity Assay, the analysis of mRNA of 238 genes was carried out, which showed a difference in the level of expression when analyzing samples of prereceptive, receptive and postreceptive endometrium.
Main results and the role of chance: The results of the studies of the level of endometrial receptivity revealed a shift in the period of the "implantation window" in 28% of patients. The results obtained make it possible to carry out the transfer of embryos in an individual order for each patient, taking into account the data on the level of receptivity of the endometrium, which makes it possible to implement the so-called Personalized Embryo Transfer (pET) principle. In 72% of cases, the study showed the "receptive" status of the endometrium. The result means that the period of the "implantation window" for a particular type of cycle falls on the moment of biopsy and this period of time is the most favorable for implantation of the embryo. For patients from this group, embryo transfer is carried out at the next repetition of the cycle option selected for the study of receptivity, while the blastocyst transfer occurs on the same day of the cycle when the endometrial biopsy was performed.
Limitations, reasons for caution: The ERA test is unique technique.
Wider implications of the findings: Using the ERA test allow to determine the state of maturity of the endometrium. Unlike other methods for receptivity of the endometrium, the ERA test allows not only to accurately determine the period of the "implantation window", but also to reliably predict its displacement to an earlier or later time.
Trial registration number: Not applicable

P-298 High prevalence of depression and anxiety in patients with endometriosis during the SARS-CoV-2 pandemic in Germany

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Study question: The aim was to assess the prevalence of self-reported symptoms of depression and anxiety and the moderating factors influencing mental symptoms during the COVID-19 pandemic.
Summary answer: Endometriosis patients were at risk of developing mental disorders during the pandemic. Associated risk factors were: reduction of the social network and the employment status.
What is known already: Endometriosis is a disease affecting up to 10% of women of fertile age. The leading symptoms are sub- or infertility and chronic pain. Additionally, the psychological impact on women´s life is enormous. Women with endometriosis show higher rates of depression, anxiety and emotional distress, and these alterations in mental health were associated with the presence of pain rather than with the diagnosis of endometriosis. Additionally, a higher level of depression was observed in women with endometriosis.
Study design, size, duration: To assess the impact of the government-imposed social distancing or quarantine on mental health, an online questionnaire was placed on internet platforms of endometriosis patients support groups between 6th and 27th April 2020. Data collection and analysis were performed anonymously. Recruitment was conducted via a direct link to the survey and an invitation to participate was distributed via the internet platforms of patients support groups.
Participants/materials, setting, methods: 274 participants answered the Patient Health Questionnaire for Depression and Anxiety (PHQ-4), which screens for depression (PHQ-2), anxiety (GAD-2) and the level of psychological distress (PHQ-4). PHQ-2 and GAD-2 scores ≥ 3 are cut-off points between normal range and probable cases of mental disorders. A PHQ-4 score above 6 is indicative for severe symptoms. We used descriptive statistics to describe the study population. Correlates of depression and anxiety were identified using multivariate logistic regressions.
Main results and the role of chance: We showed that depression and anxiety were highly prevalent in endometriosis patients during the pandemic: 46.7% and 48.2% of participants showed scale scores of ≥3 on the PHQ-2 and GAD-2 scales, respectively.

The mean PHQ-4 score was 5.72 (SD=3.21), thus endometriosis patients were achieving significantly higher PHQ-4 sores ($p<0.001$) than participants of a previously published study of the representative German population.

Risk factors for higher probability of depressive disorders were the employment status (being employed, OR 2.890, $p<0.001$), an important or severe reduction of the social network (OR 2.02, $p<0.05$), having continuous pain (OR 1.83, $p<0.05$) and high level of dysmenorrhea prior to the pandemic (OR 2.106, $p<0.05$).

Risk factors for higher probability of anxiety were the employment status (being employed, OR 2.697, $p<0.001$), an important or severe reduction of the social network (OR 3.038, $p<0.01$), and high level of dysmenorrhea prior to the pandemic (OR 1.750, $p<0.05$).

Endometriosis patients were at higher risk for developing mental health problems. The effective use of brief screening measures, such as PHQ-4, can be widely implemented even in the busy outpatient care of general practitioners and gynecologists and may help to reduce morbidity.
Limitations, reasons for caution: We used self-reports for assessment of anxiety and depression and those are susceptible to response bias, such as giving socially desirable responses. Moreover, as PHQ-4 is only a screening tool, the diagnosis must be confirmed in accordance to the appropriate DSM–V criteria.
Wider implications of the findings: A better understanding of potential mental problems in endometriosis patients during stressful events, such as the COVID-19 pandemic or other comparable difficult environmental or social circumstances, is crucial for providing an optimal patient centered care in cases of upcoming stressful events.
Trial registration number: Not applicable

P-299 Efficacy and safety of linzagolix for the treatment of severe adenomyosis: Initial results from a pilot study

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Study question: Is a once daily regimen of the GnRH antagonist, linzagolix, high-dose (200mg) for 12 weeks then low-dose (100mg) for 12 weeks, effective in severe adenomyosis?
Summary answer: After 12 weeks, there was marked shrinkage of uterine volume, regression of adenomyotic lesions and symptom improvement (pain, anemia), 24 weeks data is pending.
What is known already: Suppression of estradiol using GnRH antagonists has been shown to be an effective treatment for endometriosis and uterine fibroids. Linzagolix is an investigational, oral GnRH receptor antagonist, which dose-dependently reduces E2 levels, providing full suppression (serum E2 < 20

pg/mL) and partial suppression with once daily oral dosing of 200 mg and 100 mg, respectively. We hypothesized that a regimen of full suppression for 12 weeks followed by partial suppression maintenance therapy for 12 weeks could be effective for the treatment of severe adenomyosis.

Study design, size, duration: This was a single-center, open-label exploratory study in women with symptomatic adenomyosis confirmed by Magnetic Resonance Imaging (MRI) (EudraCT number: 2017-004-042-14). Patients were recruited from a single private clinic and infertility research unit between March 2019 to June 2020.

Participants/materials, setting, methods: Eligible patients were premenopausal women 18 to 48 years old with symptomatic uterine adenomyosis confirmed by MRI, moderate-to-severe pain and abnormal uterine bleeding. The primary measure of efficacy was the reduction in uterine volume assessed by MRI. Other endpoints included adenomyosis lesion volume, pelvic pain, haemoglobin, uterine bleeding and quality of life (EHP-30 domains: pain, control and powerlessness, emotional well-being, social support and self-image).

Main results and the role of chance: Eight (3 black and 5 white) enrolled subjects had mean±SD age 42±3 years and weight 75±19 kg. At baseline (day 2 of the cycle) all patients presented with pelvic pain, severe dysmenorrhea and heavy menstrual bleeding. In all cases, MRI showed an enlarged uterus (mean±SD volume 343±253 cm3) with severe adenomyosis characterized by heterogenous myometrium with multiple myometrial cysts. The mean±SD junctional zone was 29.0±14.2 mm. Median serum estradiol was suppressed to 12 pg/mL by 4 weeks and this was maintained up to 12 weeks. After 12 weeks, mean±SD uterine volume was 162±117 cm3, a 57±16% reduction from baseline, with marked regression of adenomyotic lesions and the junctional zone was 21.0±13.4 mm. Mean±SD overall pelvic pain score (0-10 NRS) was reduced from 8.4±1.1 at baseline to 2.4±3.4 (p=0.0035) and there were also improvements in dysmenorrhea, dyspareunia, non-menstrual pelvic pain and dyschezia scores. No subjects reported uterine bleeding between Weeks 4 to 12. Mean±SD haemoglobin was 12.1±2.0 at baseline and 12.8±1.1 at 12 weeks. Anemia at baseline (≤10g/dL) was resolved by 12 weeks. Substantial improvements were observed on each of the EHP-30 domains.

The most common side effect was the expected hypoestrogenic side effects of hot flushes, which were reported by 6/8 subjects.

Limitations, reasons for caution: This was a single-centre, open-label pilot study in 8 patients with symptomatic adenomyosis. We report the results after the first 12 weeks treatment of a high full suppression dose of linzagolix. Results after 24 weeks will further inform on the potential for a low partial suppression dose to maintain efficacy.

Wider implications of the findings: The initial results of this open-label pilot study in women with severe adenomyosis indicate that a high full suppression dose of linzagolix 200 mg is effective in reducing uterine and adenomyosis lesion size, reducing abnormal uterine bleeding and pelvic pain and improving quality of life.

Trial registration number: EudraCT number: 2017-004-042-14

P-300 Metformin: new therapeutic approach for endometriosis-associated infertility

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Study question: Could endometriosis-associated infertility be mitigated by metformin?

Summary answer: Metformin treatment restored fertility to control rates in endometriosis-induced mice that present lower fertility. No effect was observed on sham-operated mice.

What is known already: Endometriosis is a gynaecological disorder characterized by ectopic vascularized endometrial tissue growth, mainly in pelvic cavity, which provokes pain and infertility. Corroborating observations in women, endometriosis decreases oocyte quality and pregnancy success in rodents, without affecting the number of ovulations, resorption rate and fetal weight. Thus, animal models of endometriosis constitute a valuable tool to elucidate the pathophysiology of the disease and putative pharmacological therapies. Metformin is widely used for diabetes type-2 treatment, reducing glucose, oxidative stress and inflammation. Due to its antioxidant properties, metformin has shown to induce regression of endometrial implants in a rat model of endometriosis.

Study design, size, duration: B6CBA/F1 female mice were randomly divided in groups and subjected to treatment: 1-Endometriosis (n=20); 2-Sham-operated (n=12); 3-Endometriosis with metformin (n=20); 4-Sham-operated with metformin (n=20). Endometriosis was surgically induced by heterologous transplantation of endometrium from one donor in receptors from the same strain mice. Implants were confirmed and monitored by ultrasound. 50mg/kg/day of metformin was orally administrated during 3 months to Groups 3 and 4. Half of mice in each group were mated to fertility study.

Participants/materials, setting, methods: Endometriomas were monitored at 3 timepoints during the experiments. Biometric parameters of mice and number of implantation sites, fetuses and fetal weight were recorded. Fertility rates were assessed by the average number of fetuses in each group. Histological characterization of ovary, uterus, endometriomas, and peritoneal tissue at the implant site was performed by Hemathoxylin & Eosin (H&E) staining. Statistical study among groups was carried out and significant differences were considered for student t-test < 0.05.

Main results and the role of chance: A decrease of 30% of fertility rate was verified in mice with endometriosis (p=0,01); treatment with metformin was able to revert this decrease (p=0,04). Interestingly, no differences in fertility were found in sham-operated mice under metformin treatment relatively with those of group 2 (p=0,16). Although the number of absorptions observed in mice of the endometriosis group was higher, no statistical difference was reached comparatively with other groups. No biometrical differences were found between mice with endometriosis receiving metformin and those that do not receive the drug. Regarding H&E staining we verified that endometriomas showed histologically resemblances to uterus. Moreover, endometriomas from mice without treatment with metformin were dark brown, recalling for human endometriomas called "chocolate" cysts, while the endometriomas from mice who were treated with metformin were visually clearly. We postulate that these findings owes to a metformin-mediated decrease of oxidative imbalance and inflammatory response, induction of regression of endometriomas and regulation of oestrogen secretion.

Limitations, reasons for caution: Extrapolation of data from animal models to human needs caution, considering that endometriosis pattern differs between species. Also, further investigation, focused in identification of molecular targets of metformin and molecular pathways activated in endometriosis, is needed and in course.

Wider implications of the findings: With these results, we indicate metformin as a novel and safe strategy to mitigate endometriosis-related oxidative stress and indeed could be used as a valid pharmacological approach to ameliorate endometriosis-associated infertility.

Trial registration number: Not applicable

P-301 Intrauterine instillation of autologous platelet rich plasma for thin endometrium improves the outcome of frozen embryo transfer cycles

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Study question: How does the intrauterine instillation of autologous platelet rich plasma (PRP) affect the endometrial thickness and live birth rate in frozen embryo transfer cycles?

Summary answer: Intrauterine instillation of autologous PRP resulted in significant improvement in endometrial thickness. The live birth rates were satisfactory post-PRP instillation.

What is known already: Autologous Platelet rich plasma (PRP) had resulted in significant improvement in endometrial thickness, when instilled intrauterine in women with thin endometrium in FET cycles.

Study design, size, duration: A retrospective observational study was performed at a tertiary care university teaching hospital in South India. 35 women who received intrauterine autologous PRP during endometrial preparation for frozen embryo transfer from June 2017 to December 2020, were included. Patients who underwent donor oocyte recipient cycles, those with a history of tubercular endometritis, Asherman syndrome, previous intrauterine manipulations such as manual removal of placenta, and uterine anomalies were excluded.
Participants/materials, setting, methods: All the women underwent endometrial preparation in artificial cycles by depot GnRH agonist suppression and HRT (Hormone replacement therapy) was initiated by 4-6 mg of estradiol valerate and stepped up as required. Autologous PRP was offered to all women who had endometrial thickness < 7 mm on day 16 of HRT. PRP was prepared by the two-step centrifugation method and administered intrauterine by IUI catheter. The patients underwent repeat evaluation after 5 days post-PRP instillation.
Main results and the role of chance: Optimal response to PRP was considered as the attainment of an endometrial thickness (ET) ≥ 7mm after 5 days of post-PRP. 25 (71.4%) had an optimal response to PRP. There was a significant improvement in the endometrial thickness(mm) in the study participants following PRP instillation: 6.3 ± 0.6 vs. 7.1 ± 1.2; P=0.0001.The study participants were divided into two groups based on their response to intrauterine PRP instillation. Those who optimally responded to PRP were categorized as Group A and those who didn't were categorized as Group B. The study participants of both the groups were comparable by their demographic characteristics such as age, cause of infertility, indications for ART, and the dose of estradiol valerate before PRP. The dose of estradiol valerate (mg) after PRP was significantly higher in Group B compared to Group A: 19.9 ± 4.9 vs. 15.6 ± 3.9; P=0.014. A total of 26 women underwent embryo transfer and 9 (25.7%) women had cycle cancellation. Of these 22 were from Group A and 4 from Group B. The pregnancy, clinical pregnancy, miscarriage and live birth rates were 36.3%(8/22) and 25% (1/4); 31.8% (7/22) and 25% (1/4); and 31.8% (7/22) and 25% (1/4), respectively.
Limitations, reasons for caution: As the study was retrospective in nature and the PRP was offered only in patients who had consented, there was a significant bias. Hence the results of the study should be interpreted with caution. Further large prospective RCTs (Randomised controlled trials) are required to confirm our findings.
Wider implications of the findings: Autologous PRP may enhance the response to the estrogen preparations. It may produce satisfactory live birth rates and reduce cycle cancellations in a reasonable proportion of patients with thin endometrium in FET cycles. However, these findings should be confirmed by dose finding clinical trials, and studies involving a comparison group.
Trial registration number: not applicable

P-302 Levonorgestrel-releasing intrauterine device (LNG-IUD) for symptomatic endometriosis following surgery: a Cochrane systematic review

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Study question: Does levonorgestrel-releasing intrauterine device (LNG-IUD) improve post-operative outcomes for endometriosis when compared to other systemic hormonal treatments or no additional treatment?
Summary answer: We are uncertain whether LNG-IUD has an impact on dysmenorrhoea when compared to no postoperative treatment or post-operative GnRH agonists (GnRH-a).
What is known already: Endometriosis is a condition characterised by the presence of ectopic deposits of endometrial-like tissue outside the uterus, usually in the pelvis; inducing a chronic inflammatory response which can lead to pelvic pain and infertility. Various treatment options exist including surgical treatment, ovarian suppression therapy, or a combination of these strategies. The impact of laparoscopic treatment on overall pain is uncertain and a significant proportion of women will require further surgery. Therefore, adjuvant medical therapies such as LNG-IUD have been considered to reduce treatment failure and recurrence of symptoms.
Study design, size, duration: A Cochrane systematic review and meta-analysis was performed. Electronic searches of the Cochrane Gynaecology and Fertility Specialised Register of Controlled Trials, CENTRAL, MEDLINE, EMBASE, PsycINFO, CINAHL and Epistemonikos were conducted to January 2021 for relevant randomised controlled trials (RCTs). Two independent authors screened studies and extracted data. Risk ratios (RR) were calculated for dichotomous data and standardised mean differences (SMD) for continuous data, with 95% confidence intervals (CI). Heterogeneity was examined via the I^2 statistic.
Participants/materials, setting, methods: Participants: women undergoing surgical treatment for endometriosis without hysterectomy
Intervention: LNG-IUD insertion within three months of surgery
Comparison: No postoperative treatment, placebo IUD or any other systemic treatment
Primary outcome: overall pain
Secondary outcomes: improvement of the most troublesome symptom, dysmenorrhoea, quality of life, satisfaction with treatment and adverse events. Primary analysis was conducted on data per woman randomised.
Main results and the role of chance: Four RCTs were included, with a total of 157 women. Five studies are awaiting classification and one is an ongoing study. We corresponded with original study authors to clarify missing outcome data. No studies reported on overall pain or improvement in the most troublesome symptom. We await study author clarification on quality of life and treatment satisfaction data. We are uncertain whether LNG-IUD improves dysmenorrhoea compared to no postoperative treatment at 12 months. Data on this outcome were reported on by 2 RCTs, but were expressed as median and inter-quartile range and so meta-analysis was not possible (RCT 1: delta of median visual analog scale (VAS) 81 versus 50, p = 0.006, n = 55; RCT 2: fall in VAS by 50 (35–65) p=0.012 versus 30 (25– 40), p = 0.021, n = 40). We are uncertain whether compared to post-operative GnRH-a, LNG-IUD affects rates of dysmenorrhoea at 6 months (VAS SMD 0.79, 95% CI -0.08 to 1.67, p=0.08, one RCT, n = 22, very low quality evidence). Various adverse events with LNG-IUD were reported including irregular bleeding and weight gain. However, due to a lack of raw data and comparable studies, we were unable to undertake meta-analysis.
Limitations, reasons for caution: The major limitation of this systematic review was that there were insufficient studies reporting on our prespecified outcomes, including our primary outcome. In addition, the included studies were not all of high quality with limited long-term follow-up.
Wider implications of the findings: This systematic review highlights the paucity of RCTs reporting outcomes included in the new core outcome set for endometriosis research. Further high-quality RCTs are needed to assess post-operative adjuvant hormonal therapy and these should prioritise investigating key endometriosis outcomes such as overall pain, quality of life and treatment satisfaction.
Trial registration number: Not Applicable

P-303 Similar long term recurrence rates with cystectomy and CO2-laser vaporization for endometrioma: a retrospective study

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Study question: Is there a difference in recurrence rate of endometrioma(s) after cystectomy versus CO2-laser vaporization of the cyst wall?
Summary answer: Similar rates of imaging based recurrence or need for reintervention for endometrioma were observed after cystectomy versus CO2-laser vaporization.
What is known already: Surgical treatment of endometrioma(s) is mainly performed by 2 types of procedures: cystectomy and ablation. When performing surgery for endometrioma(s), a balance should be made between minimal destruction of normal ovarian cortex and maximal completeness to avoid (early) recurrence.

Previous studies have shown that cyst recurrence rates were higher with ablation using bipolar current than after cystectomy. However, only 2 groups

have evaluated recurrence rates after cystectomy versus CO2 laser vaporization and found no difference with extended follow-up. Furthermore, ablation with CO2 laser may be less invasive than conventional cystectomy with increased preservation of antral follicles in favor of ablation.

Study design, size, duration: Single-center retrospective study on data of 271 patients operated between January 2010 and December 2014.

Participants/materials, setting, methods: Women of reproductive age (18-45 years), undergoing CO2 laser laparoscopic excision of any rAFS-stage endometriosis with at least one endometrioma, were eligible for the study. All 271 included patients were treated in a tertiary referral center for endometriosis of a University Hospital, and underwent complete CO2-laser laparoscopic surgery for endometrioma(s). 155 underwent cystectomy, 77 CO2 laser vaporization, and 46 a mixed technique.

Main results and the role of chance: The mean duration of follow-up was 58 months. Primary outcome studied was the comparison of recurrence rates between cystectomy and vaporization; secondary outcomes included pregnancy rate and ovarian reserve testing. Recurrence was defined as either imaging based (i.e. cyst recurrence identified at ultrasound and/or MRI) or need for reintervention for suspected cyst recurrence. Imaging based recurrence was reported in 9.92% of patients (n = 12/121) treated with cystectomy and in 11.76% of patients (n = 6/51) who underwent a CO2 laser vaporization (p = .62). The need for reintervention for endometrioma(s) was also similar in both groups, with a rate of 3.23% (n = 5/155) after cystectomy and 4.29% (n = 3/70) after CO2 laser vaporization (p = .567). No difference was seen regarding AMH drop pre- versus postoperatively (p=.233).

The 2 study groups were similar, except for the mean cyst diameter, which was higher in the cystectomy group (42.36 ± 25.49 mm) compared to the CO2 laser vaporization group (31.7 ± 26.98 mm) (p = <.001). This suggests that smaller endometriomas might be more likely to undergo CO2 laser vaporization.

Limitations, reasons for caution: The retrospective character of the study may induce information bias concerning the registration of recurrence. Moreover, regarding the evaluation of imaging-based recurrence, a selection bias cannot be excluded, because most likely only patients complaining about pain would be referred for an ultrasound or planned for a reintervention.

Wider implications of the findings: In this study, similar rates of recurrence for endometrioma(s) were observed after cystectomy versus CO2-laser vaporization. Since previous studies suggested that CO2-laser vaporization may cause less damage to the adjacent ovarian tissue, we consider this a valuable alternative technique, especially for women with a future child wish.

Trial registration number: S59032

P-304 The endometrial preparation protocol does not affect the live-birth-rate after vitrified-warmed euploid single blastocyst transfers: an analysis of 1884 procedures

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Study question: Is the live-birth-rate (LBR) different when comparing artificial (AC) and modified-natural (M-NC) cycle for endometrial preparation to vitrified-warmed euploid blastocyst transfer?

Summary answer: The LBR after vitrified-warmed euploid blastocyst transfer seem independent of the endometrial preparation administered.

What is known already: Only the transfer of a competent embryo on a receptive endometrium might result in successful implantation. Three main protocols for endometrial preparation to vitrified-warmed embryo transfer exist: NC, M-NC, and AC. None among them, though, has been shown more appropriate than the others to date, especially since, only in a few studies, the analysis was restricted to single euploid blastocyst transfers to limit the impact of embryonic issues on implantation. In conclusion, no clear consensus exists and the choice is still largely based on menstrual/ovarian cycle characteristics and patient's needs.

Study design, size, duration: All first vitrified-warmed single euploid blastocyst transfers performed between April-2013 and March-2020 were included in the analysis. Endometrial preparation was conducted with either an AC (N=1211) or a M-NC (N=673). The protocol was chosen based on patients' logistical reasons. The primary outcome was the LBR per transfer. Sub-analyses based on blastocyst quality and day of development were conducted. Birthweight, gestational age, gestational and perinatal issues were secondary outcomes.

Participants/materials, setting, methods: AC: oral estradiol-valerate 3-times/day from day2-3 of the cycle until the endometrial thickness reached ≥7mm, then 600 mg/day of micronized progesterone. The transfer was conducted on day6 of progesterone administration. M-NC: an intramuscular dose of 10,000IU hCG was administrated when the leading follicle was >17 mm and the endometrium was thicker than 7mm and trilaminar, plus 400 mg/day of micronized-progesterone as luteal phase support starting 36–40hr post-hCG. The transfer was conducted on day7 after trigger.

Main results and the role of chance: The two groups were similar for maternal age at retrieval (38.0±3.3yr) and transfer (38.3±3.3yr), reproductive history, embryological outcomes of the IVF cycle, body-mass-index, basal hormonal levels, and blastocyst features (Gardner's classification: AA=73%, AB/BA=11%, BB/AC/CA=8%, CC/BC/CB=8%; day5=48%, day6=47%, day7=5%). The LBR was 46.7% (N=565/1211) and 49.9% (N=336/673) after AC and M-NC, respectively, resulting in an odds-ratio 1.14, 95%CI:0.94-1.37. The absence of significant differences was confirmed also when adjusted for blastocyst quality and day of full-development (1.16, 95%CI:0.96-1.41). Among the 565 and 336 deliveries, the birthweight was similar (3290.3±470.7 versus 3251.7±521.5 g, Mann-Whitney-U-test=0.5), the gestational age was similar (38.5±1.7 versus 38.4±1.9 weeks, Mann-Whitney-U-test=0.5). Also, the rates of newborns who were normal (81% versus 82%), large (8% versus 9%), and small (11% versus 9%) for gestational age were similar (Chi-squared-test=0.5). The rates of patients experiencing gestational (6% versus 7%) and/or perinatal issues (3% versus 3%) were also similar (Fisher's-exact-tests=0.4).

Limitations, reasons for caution: This is a retrospective study conducted in poor prognosis patients indicated to preimplantation genetic testing for aneuploidies. Future randomized controlled trials and cost-effectiveness analysis are desirable, as well as studies in different patient populations. Lastly, each gestational/perinatal issue shall be analyzed *per se* (e.g. different placentation disorders).

Wider implications of the findings: The absence of clinical and perinatal differences between the two protocols for endometrial preparation supports the adoption, whenever needed, of AC. This approach, in fact, allows a higher flexibility in patients' and daily workload management.

Trial registration number: None

P-305 The expression of innate immune factors in the eutopic endometrium of women with endometriosis

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Study question: Is the expression of IL-17A and antimicrobial peptides (AMPs) altered in endometriosis?

Summary answer: IL-17A protein levels were similar in the endometrium of women with and without endometriosis but the expression of several AMPs was reduced in endometriosis.

What is known already: Chronic inflammation and aberrant immune signalling in the eutopic endometrium underlies the pathophysiology of endometriosis. Endometrial IL-17 levels have previously been shown by our group to be negatively correlated with successful pregnancy. IL-17 has also been associated with endometriosis. Among its immunomodulatory functions, IL-17 regulates the expression of several antimicrobial peptides (AMPs), highly conserved proteins that are involved in the innate immune response. Despite the well accepted paradigm of immune dysregulation in endometriosis, surprisingly little is known

about the relationship between endometrial IL-17, AMPs and endometriosis, particularly in the context of infertility.
Study design, size, duration: This was a prospective cohort study. Endometrial biopsy samples were collected at the time of endometrial scratch testing, or during elective laparoscopy, over a 15-month period. The aim was to evaluate and compare IL-17A protein levels and the expression of specific AMPs (SLPI, elafin, beta-defensin 1, S100A7, S100A8, S100A9) [MW1] , in the endometrium of patients with endometriosis, and those without the disease.
Participants/materials, setting, methods: Thirty-two patients were recruited for the study, with 26 included in the final analysis. Biopsies were obtained at the time of planned endometrial scratch (mid-luteal phase) prior to ART (n=7), or at the time of laparoscopy (n= 19). RNA was extracted and Q-PCR analysis for AMP transcript levels was performed. ELISA was carried out to quantify levels of IL-17A in endometrial tissue in a subgroup (n=17). Main results and the role of chance: In our cohort IL-17A protein levels were not significantly different in the endometrium of patients with disease compared to those without (p=0.3636). The expression of the AMPs elafin, SLPI, BD1 and S100A9, as measured by a Q-PCR transcript profiling approach, were found to be significantly reduced in the eutopic endometrium in cases of endometriosis as compared to cases without endometriosis. There was no significant difference in AMP expression between disease stages. No demonstrable difference in IL-17A protein levels, or AMP expression was detected between the proliferative and secretory phases of the menstrual cycle.
Limitations, reasons for caution: Numbers may not have been large enough to fully evaluate the impact of endometriosis stage or the effect of cycle phase. While all patients with endometriosis had surgical confirmation of disease, not all patients in the control cohort had a laparoscopy, though they had no clinical features suggestive of endometriosis.
Wider implications of the findings: Our findings indicate that the innate immune environment of the eutopic endometrium is altered in endometriosis. Endometrial expression of AMPs was diminished in endometriosis, independent of disease stage. This work supports the model of dysregulated innate immune system in endometriosis, and reveals AMPs as novel immune players in disease pathogenesis.
Trial registration number: not applicable

P-306 Premenopausal women with a diagnosis of endometriosis have a significantly higher prevalence of a diagnosis or symptoms suggestive of restless leg syndrome; prospective cross-sectional study

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Study question: Are women who report a previous surgical diagnosis of endometriosis more likely to have a diagnosis or symptoms suggestive of restless leg syndrome (RLS)? Summary answer: Women who reported a prior surgical diagnosis of endometriosis, have a significantly higher prevalence of prior formal diagnosis of RLS or symptoms suggestive of RLS.
What is known already: Endometriosis and RLS are both chronic conditions that can negatively affect a woman's quality of life. A higher prevalence of RLS is seen in women and particularly in those who are pregnant, suggesting a possible ovarian hormonal influence. Endometriosis is a common (affecting 1 in 10 women) oestrogen driven gynaecological condition, and the prevalence of RLS in women with symptoms or a diagnosis of endometriosis is unknown.
Study design, size, duration: This was a prospective, cross-sectional, observational self-completed questionnaire study. Questionnaires were distributed to 650 women under 50 years of age attending the gynaecology out patient's department at the Liverpool Women's hospital from October 2017 to January 2018.
Participants/materials, setting, methods: 584 women returned the voluntary, anonymous questionnaires, which assessed RLS-associated (The International Restless Leg Syndrome Study Group rating scale) and endometriosis-associated (modified-British Society of Gynaecological Endoscopists pelvic pain questionnaire) symptoms. The final dataset included 465 completed questionnaires.
Main results and the role of chance: The overall response rate for this study was high (90%, 584/650). Women who reported a prior surgical diagnosis of endometriosis had a greater risk of having a prior formal diagnosis of RLS (OR 4.82, 95% CI 1.66,14.02) and suffering RLS symptoms (OR 2.13, 95% CI 1.34-3.39) compared with those without a diagnosis. Women with either a formal diagnosis or symptoms associated with endometriosis have a significantly increased risk of having either a formal diagnosis or symptoms suggestive of RLS (OR 2.49, 95% CI 1.30, 3.64).

In women suffering with endometriosis-associated symptoms, the cumulative endometriosis-associated symptom scores demonstrated a modest positive correlation with RLS severity scores (r=0.42 95% CI 0.25 to 0.57).
Limitations, reasons for caution: The anonymous, voluntary self-completed questionnaire findings were not confirmed directly using medical records. However, our questionnaire that was piloted for acceptability prior to the study, allowed collation of information directly from women, regarding their clinically relevant symptoms that are important in diagnosing RLS.
Wider implications of the findings: This is the first study highlighting an association between the symptoms relevant to these two chronic conditions, which may help in facilitating the discovery of novel therapeutic targets relevant to both. The simultaneous treatment of these conditions could potentially lead to improvement in overall quality of life for these women.
Trial registration number: NA

P-307 Fatty acid degradation during in vitro decidualization of human endometrial stromal cells

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Study question: Is the activity of the β-oxidation pathway, involved in the degradation of fatty acids, modified during *in vitro* decidualization of human endometrial stromal cells (HESC)?
Summary answer: The level of expression of fatty acid´s transporters suggests that the activity of the mitochondrial β-oxidation pathway is increased during *in vitro* decidualization of HESC.
What is known already: The differentiation of endometrial stromal cells (ESC), named decidualization, is essential for the proper formation of the materno-fetal interphase. One important feature of decidualization is the increased glucose consumption. In the endometrium, glucose is incorporated into ESC by glucose-transporters (GLUT). Fatty acids are another important energy source in living cells. Fatty acids are transported into mitochondria by the carnitine-palmitoyl-transferases 1 and 2 (CPT1 and 2) and are degraded there through the β-oxidation pathway. It has been described that the inhibition of CPT1 affects ESC decidualization. However, it is unknown whether the turn-over of fatty acids degradation is modified during decidualization.
Study design, size, duration: This study was performed using primary HESC. Endometrial biopsies (mid-late proliferative-phase) were obtained from healthy-regularly-cycling women (33.6±2.2 years-old) after written informed consent was obtained (protocol approved by Ethics committee no. S-239/2005). HESC were decidualized (D) *in vitro* with a decidualization-cocktail (containing: medroxyprogesterone acetate, estradiol and 8-Bromo-cyclic adenosine monophosphate) for 6 days. Non-decidualized (ND) controls were treated with vehicle solutions. Cell-culture supernatant and cell extracts were collected for the evaluation of protein/gene expression and metabolite content.
Participants/materials, setting, methods: Decidualization was evaluated by measuring prolactin (PRL) protein levels in cell-culture supernatant (mU/l). Changes in mRNA expression levels of *GLUT1*, *CPT1A* and *CPT2* were evaluated by real-time polymerase chain reaction (RT-PCR). Analysis was performed by the ΔΔCt method (internal control: *RPLP0*) (fold change -FC- in D compared to ND cells). Contents of acylcarnitines were evaluated by Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS) (nmol/mg of total

protein). N=5, mean±SEM. Paired Student's t-test was used for statistical analysis.

Main results and the role of chance: PRL protein levels in cell-culture supernatant were significative increased in HESC treated with the decidualization-cocktail compared to ND cells (ND 16.80±0.73 mU/l; D 684.20±219.80 mU/l, *p<0.05). This result confirmed the decidualized state of HESC upon *in vitro* treatment with the decidualization-cocktail. Additionally, the mRNA expression level of *GLUT1* was highly upregulated in D compared to ND cells (FC 10.02±2.90, ***p<0.001), consistent with the increase in glucose consumption characteristic of decidualization. Once confirmed the decidualized state of HESC, the mRNA expression levels of *CPTA1* and *CPT2* were evaluated. The mRNA expression levels of both fatty acid´s transporters were upregulated in D compared to ND cells (*CPTA1*: FC 1.84±0.44, **p<0.01; *CPT2*: FC 2.04±0.49, **p<0.01). Finally, the content levels of different acylcarnitines, intermediate metabolites of the -oxidation degradation of fatty acids, were evaluated. The concentrations of acetyl- (C2) and butyryl- (C4) acylcarnites were decreased in D compared to ND cells [(C2: ND 1.37±0.10 nmol/mg of total protein; D 1.06±0.20 nmol/mg of total protein, *p<0.05), (C4: ND 0.03±0.01 nmol/mg of total protein; D 0.01±0.00 nmol/mg of total protein, *p<0.05)]. The content levels of other intermediate acylcarnitines measured from cell extracts had no differences between D and ND cells (p>0,05).

Limitations, reasons for caution: This study was performed *in vitro* using primary HESC treated with a decidualization-cocktail. The interconnection of different metabolic pathways within a living cell is very complex. Further studies are necessary to define whether the different intermediate metabolites of the mitochondrial -oxidation pathway are being used by related-metabolic pathways during decidualization.

Wider implications of the findings: The regulation of the energy metabolism and its interconnection with other important intra-cellular metabolic pathways is of great importance for cellular function. Our results contribute to highlight the importance of the regulation of fatty acids degradation during decidualization. Further insights into HESC metabolism could facilitate the improvement of women´s health.

Trial registration number: not applicable

P-308 Fertility preservation in endometriosis: Appraising the factors affecting the ovarian response

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Study question: To estimate the factors affecting the ovarian response in women with endometriosis who seek fertility preservation.

Summary answer: AMH was the most important predictor of ovarian response.

What is known already: Endometriosis is a chronic disease characterised by the presence of endometrial tissue outside the uterine mucosa. This condition affects up to 10% of reproductive-aged women and up to 50% of women with infertility. Infertility in patients affected with endometriosis has been thought to stem mainly from the inherent effect of implants on the ovarian reserve or by the distortion of the female upper reproductive tract organs in the late stages of the disease. Women diagnosed with endometriosis should be counselled about family planning however, the data available to guide these patients regarding fertility preservation or oocyte freezing is scarce.

Study design, size, duration: A Retrospective cross-sectional study was conducted from July 2017 to May 2020 at a university-affiliated medical center. Patients who had been treated in the endometriosis clinic and underwent controlled ovarian stimulation and oocyte retrieval for fertility preservation, filled an online questionnaire crossed reference with electronic chart analysis related to patient data and fertility preservation cycles.

Participants/materials, setting, methods: Eighty-one patients were included and categorized into two groups according to the number of oocytes retrieved: 0–5 (Group A, n = 26) low ovarian response and 6 or more oocytes (Group B, n = 55).

Main results and the role of chance: The severity and *duration of the disease* so as the symptoms indicative of deep infiltrating endometriosis, were not associated with reduced numbers of vitrified oocytes. The presence of deep infiltrating implants was not associated with numbers of vitrified oocytes (44.4% vs 70.6%, p = 0.08). More Patients who underwent surgical interventions, had significantly lower ovarian responses compared to women who had no intervention (95.2% vs 61.5%, respectively, p = 0.005). A multivariate logistic regression adjusted for the number of oocyte vitrified revealed that anti-Müllerian hormone (AMH) level below 0.8 ng/ml was the only factor significantly associated with low ovarian response, with an adjusted odds ratio of 13.40 (2.02-157.27, p = 0.015). Limitations, reasons for caution: The size of our cohort is relatively small for the number of covariates, reducing the impact of our results when put on an international scope and the lack of information regarding the return rate of patients who had their oocytes vitrified in the attempt of achieving a pregnancy.

Wider implications of the findings: We believe that since the practice of FP for endometriosis is relatively new and there is a substantial lack of data, this cohort represents an important contribution to existing literature by extracting data from medical files and cross-referencing them with existing data for fertility specialists, patient encounters, and hospital registries.

Trial registration number: not applicable

P-309 Effectiveness of intrauterine infusion of platelet-rich plasma (PRP) vs. granulocyte colony-stimulating factor (G-CSF) in women with thin endometrium undergoing assisted reproduction

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Study question: To compare the effect of intrauterine infusion of platelet-rich plasma (PRP) and granulocyte colony-stimulating factor (G-CSF) on endometrial thickness, clinical pregnancy rate and live-birth rate.

Summary answer: According to our research, PRP-therapy has more positive results in pregnancy rates. Nevertheless, there is still limited data to answer all questions completely.

What is known already: Thin endometrium is defined as endometrium thickness <7 mm or <8 mm on the day of human chorionic gonadotropin (HCG) administration, which negatively affects the onset of pregnancy during natural fertilization and in assisted reproduction.

Endometrial thickness may impact pregnancy and live-birth rates in fresh and frozen IVF cycles but, currently, there is minimal evidence to support any specific protocol to significantly improve pregnancy outcomes in patients with thin endometrium. Therefore, we decided to compare effects of two methods: transvaginal intrauterine perfusion of G-CSF for infertile women with thin endometrium in IVF cycles and intrauterine infusion of PRP.

Study design, size, duration: We conducted a systematic review and meta-analysis by PRISMA checklist. PubMed, The Cochrane Library, ClinicalTrials.gov and Google Scholar were searched electronically until 2021 using key words: "G-CSF", "PRP", "endometrium". We included published and unpublished randomized clinical trials (RCT) and non-randomized clinical trials in English which include min.10 patients.

Participants/materials, setting, methods: Participants: female infertile patients of reproductive age with thin endometrium (≤ 7 mm) in embryo transfer cycles. Exclusion criteria: history of any chromosomal or genetic abnormalities and hematological disorders. Outcomes – endometrial thickness, pregnancy and live-birth rates.Risk of bias assessment was held using The Cochrane Handbook and The Cochrane collaboration tools. RoB 2 tool was used for randomized trials and ROBINS-I tool for non-randomized trials. For quantitative synthesis RevMan 5.4 was used to conduct meta-analysis.

Main results and the role of chance: Literature search resulted in 144 potentially relevant publications initially (Google Scholar: 126, PubMed: 1, Clinicaltrials: 14, Cochrane: 3). These publications were checked for titles and abstracts, duplicates were removed and 7 publications were selected. After evaluating the selection criteria, 2 articles were excluded. As a result, 5 articles remained for qualitative and quantitative synthesis, including 2 randomized controlled trials (RCT), 2 retrospective cohort studies, and 1 prospective cohort study. 403 patients participated in selected studies (the PRP group included 205 patients, and the G-CSF group - 198 patients). The primary quantitative analysis (meta-analysis) is aimed at comparison of endometrial thickness in patients receiving PRP therapy and patients receiving G-CSF. Two RCTs were included in this meta-analysis. (RR = 1.07, 95% CI: 0.81 to 1.43, P = 0.63). The heterogeneity for this comparison was 0%. Consequently, both options are equally altered by the thickness of endometrium.

The secondary analysis compared clinical pregnancy rates in patients receiving PRP therapy and G-CSF. 4 studies were eligible for this synthesis. (RR = 1.36, 95% CI: 1.06 to 1.76, P = 0.02). The heterogeneity for this comparison was 55%. PRP was significantly more effective.

Limitations, reasons for caution: Firstly, only 5 publications were found for the entire time. There are not enough well-conducted studies for more accurate analysis. Secondly, according to risk of bias assessment most of included studies had moderate concerns. Finally, there is no sufficient data to judge about live-birth rates after both types of treatment.

Wider implications of the findings: Thin endometrium negatively affects the onset of pregnancy in assisted reproduction.Based on our meta-analysis, PRP therapy has a considerable effect on pregnancy rates in patients with thin endometrium in comparison with G-CSF.Currently, there is minimal evidence to support any specific protocol for improving IVF outcomes in women with thin endometrium.

Trial registration number: PROSPERO 2020 CRD42020222075

P-310 Women with endometriosis achieve live birth after a similar number of embryo transfers independent of the endometriosis subtype

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Study question: How many embryo transfers are needed to achieve the first live birth in women with endometriosis depending on disease localisation?

Summary answer: The number of transfers needed to achieve live birth in women with endometriosis is independent from the disease's subtype.

What is known already: Infertility is one of the leading symptoms in women with endometriosis. Endometriosis is also known to negatively impact in-vitro fertilization (IVF) outcome. A reduction of oocyte yield, especially of mature oocytes in women with endometrioma (OMA) and deep infiltrating endometriosis (DIE) has been shown. Inflammatory processes possibly affect folliculogenesis and oocyte development, maybe impeding embryo development and implantation. In contrast, even with fewer retrieved oocytes per cycle live birth rate was not affected. However, it is currently unknown if specific endometriosis subtypes could differentially affect IVF success. This would be relevant for a more targeted counseling regarding the treatment success.

Study design, size, duration: This is a single-center cohort study including women (N=229) with embryo transfer cycles leading to live birth at the Bern University Hospital between 2010 and 2017. We only included women until they achieved the first live birth at our center. Participants/materials, setting, methods: We included 86 women with endometriosis and 143 women with male factor infertility serving as comparison group. We collected patient characteristics, details from the endometriosis surgery and reproductive treatment and outcomes from medical reports. We hierarchically classified the dominant endometriosis localizations as follows: deep infiltrating endometriosis (DIE, N =21) > ovarian endometriosis (OMA, N=35) > superficial peritoneal endometriosis (SUP, N = 30). We compared the number of embryo transfers needed to achieve a live birth.

Main results and the role of chance: Women with endometriosis were older (34.5 ± 3.9 years) than women from the control group (33.4 ± 3.9), p=0.03. Body-mass-index, previous parity or Anti-Mullerian hormone level did not differ between the groups SUP, OMA, DIE or the comparison group. The number of necessary embryo transfer cycles to achieve a live birth did not differ between women with SUP (3.4 ± 2.6 embryo transfers), OMA (2.9 ± 2.0), DIE (3.0 ± 2.4) and the comparison group (2.9 ± 2.2), p=0.59. IVF is beneficial in women with endometriosis, especially when OMA and/or DIE affect mobility of tubes and ovaries and spontaneous pregnancy is unlikely or impossible. This could account for the equal number of transfer cycles needed to achieve a live birth in women with OMA compared to the comparison group, even though the women with endometriosis were of older age. This is reassuring when counseling women with endometriosis.

Limitations, reasons for caution: We did not calculate cumulative pregnancy rate per cycle, because in Switzerland IVF treatment is at the patient's own cost and therefore biased. We wanted to include all women with endometriosis, so fresh and thawing cycles were included. Women from the comparison group had no surgical exclusion of endometriosis.

Wider implications of the findings: Our study suggests that the endometriosis subtype does not determine the embryo transfer success rate after IVF and therefore is less relevant for counseling. Individualized anti-inflammatory treatment before embryo transfer might positively affect the cycles' outcome. Studies with a larger sample are required to be more conclusive on this issue.

Trial registration number: BASEC 2015-00235

P-311 Association between serum sex hormones (progesterone and estradiol) and endometrial morphology during the mid-luteal phase

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Study question: Is there a relationship between the serum progesterone and estradiol levels and certain morphological characteristics of human endometrium during the mid-luteal phase?

Summary answer: Serum progesterone is associated with the stromal edema and the abundance and size of basal vacuoles in the endometrium of women during the mid-luteal phase.

What is known already: Progesterone and estrogen are essential hormones that are necessary to prepare the endometrium for pregnancy. Their serum concentrations during the mid-luteal phase are important criteria for prediction of successful embryo implantation. In addition, a variety of endometrial morphological markers, such as the presence of pinopodes, subnuclear and supranuclear vacuoles, glandular secretion, and stromal edema have been applied for determination of the window of implantation and endometrial receptivity. However, the relationship between these endometrial morphological characteristics and serum levels of progesterone and estradiol is still scarcely studied.

Study design, size, duration: This is an observational study of 98 women, 25 to 46 years of age (mean 37 years), who had a blood sample and an endometrial biopsy during the mid-lutheal phase (LH+7) in a natural cycle. The study was conducted between August 2020 and November 2020.

Participants/materials, setting, methods: Serum progesterone and estradiol were measured by electrochemiluminescence immunoassay (*ECLIA*) on the *Cobas* e411 analyser (Roche Diagnostics, Germany).

The following endometrial morphological characteristics were assessed using light microscopy: (1) basal vacuoles (mean size and percentage of vacuolated glandular cells) (2) apical vacuoles (mean size and percentage of vacuolated glandular cells), (3) pinopodes (percentage of luminal epithelium covered in pinopodes), (4) glandular intraluminal secretion (6-level scoring system), (5) stromal edema (6-level scoring system).

Main results and the role of chance: The serum progesterone levels ranged between 0.39 and 145.3 ng/ml, with a median of 24.36 ng/ml. The serum estradiol levels varied between 26.91 and 842.89 pg/ml with a median of 124.75 pg/ml. The percentage of cells with basal vaculoles ranged from 0 to 90 %, with a median of 38.57%, apical vacuoles (0-50 %, 16.83%), pinopodes (0-80 %, 23.87%), glandular intraluminal secretion (0-80 %, 28.57%), and stromal edema (1-6, 1.42).

To examine the association between the serum progesterone and estradiol and the studied endometrial morphological characteristics, the Spearman's Rho Correlation coefficient for non-paramentric data was used. No correlation was found between serum estradiol levels and the studied morphological variables (p>0.05). In contrast, the serum progesterone concentration showed a significant negative correlation with the percentage of glandular epithelial cells with basal vacuoles (R= - 0.28; p=0.03), the mean size of the basal vacuoles (R= - 0.24; p=0.5) and a significant positive correlation with the stromal edema (R=0.34; p<0.01).

Limitations, reasons for caution: The study was limited in sample size.

Wider implications of the findings: The results of this study revealed that serum progesterone is more strongly associated with the occurrence of certain endometrial morphological characteristics during the mid-luteal phase than serum estradiol. These findings are valuable for development of new methods for accurate determination of the window of implantation.
Trial registration number: not applicable

P-312 Clinical applications of platelet-rich plasma in poor endometrium patients with adenomyosis

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Study question: Does the intrauterus application of platelet-rich plasma (PRP) allow improving the thickness of endometrium in patients with adenomyosis: comparative characteristics of the PRP-techniques?
Summary answer: The endometrial thickness in poor-endometrium-patients is increased after subendometrial PRP- injection comparing with the endometrium size after PRP- irrigation of the uterine cavity (P<0.05).
What is known already: The endometrium plays an important role in achieving optimal outcomes of assisted reproductive technologies. PRP is a novel method that is used in reproductive medicine to improve the IVF outcome. The mechanisms of PRP have not been completely elucidated, but laboratory studies have shown that the high concentration of growth factors in PRP can potentially speed up the healing process. Recently, the intrauterine PRP-infusion and the subendometrial PRP-injection during the hysteroscopy have been described as a way to promote endometrial growth and receptivity.
Study design, size, duration: Totally 64 patients with adenomyosis and poor endometrium (≤ 6 mm) were divided into three experimental groups depending on PRP administration: intrauterine PRP-infusion, subendometrial PRP-injection and standard hormonal therapy of adenomyosis without PRP application. The period of study – December 2019 – November 2020. The study's protocol was approved by the Center's IRB.
Participants/materials, setting, methods: Intrauterine PRP-infusion was performed for 23 patients with age 34.2±3.6 y.o. (Group 1). Subendometrial PRP-injection was applied for 16 patients with the middle age 32,6±2,4 y.o. (Group 2). Additional hormonal therapy was carried out for patients from Groups 1, 2. The third control Group 3 consisted of 25 adenomyosis women with the middle age 35.7±4.1 y.o. Only the hormonal therapy was performed for patients of the control group.
Main results and the role of chance: The procedure of intrauterine PRP-infusion was done using the intrauterine catheter on the 8th and 12th days of the f hormone replacement therapy. Subendometrial PRP-injection was performed during the hysteroscopy on the 8th-10th day of the previous menstrual cycle. The control of the endometrium size was done by ultrasound examination. T-test was used for data analysis. P <0.05 was considered statistically significant. After subendometrial PRP-injection, the average endometrium thickness was significantly higher comparing with the control (8.7±1.1mm vs. 5.8±0.8mm) (Student t-test t=2.13, P = 0.04). PRP-infusion also showed significantly strong positive result comparing with the control group (8.3±0.9mm vs. 5.8±0.8mm) (Student t-test t=2.08, P = 0.043). There is the tendency that subendometrial PRP-has a greater impact on endometrium size comparing with the PRP-infusion. Further investigations should be done.
Limitations, reasons for caution: Patients with Asherman's syndrome were excluded from experiment.
Wider implications of the findings: It is not clear how the intrauterine administration of PRP may act to affect endometrial thickness. Results from studies on the role of endometrial thickness on implantation and live births are contradictory. There is the urge for well-designed randomized studies to improve our knowledge on PRP in reproductive medicine.
Trial registration number: -

P-313 Endometrial receptivity analysis for personalized embryo transfer in patients with recurrent implantation failure: a retrospective analysis of a Chinese cohort

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Study question: To quantify the effectiveness of endometrial receptivity analysis (ERA)-guided personalized embryo transfer (pET) in Chinese women.
Summary answer: ERA-guided pET may remarkably improve pregnancy and implantation rates among Chinese women with Recurrent implantation failure (RIF).
What is known already: RIF is a major cause of infertility, and endometrial receptivity is widely accepted to impact implantation failure. Precision prediction of the WOI, the time when the endometrium is most receptive to the implantation of the embryo, is, therefore, of great significance to improve implantation prospects. Previous studies have shown the effectiveness of ERA for the prediction of the WOI, and how pET, timed by ERA, improves implantation and pregnancy rates; however, the efficacy of ERA-guided pET remains unknown for Chinese women.
Study design, size, duration: Patients in Chengdu Xi'nan Gynecology Hospital (Chengdu, China) who were undergoing frozen embryo transfer (FET) at the blastocyst stage on day five or day six during the period from November 2019 through September 2020 were recruited for this study. A total of 145 eligible patients were included in the study and assigned to the ERA group (n = 67) or the control group (n = 78). Clinical pregnancy outcomes were compared between the two groups.
Participants/materials, setting, methods: Endometrial specimens were collected the from ERA group. Total RNA was extracted from endometrial specimens, the transcriptomic sequencing data were processed using RNA-Seq and the endometrial receptivity status was assessed by the ERA predictor. The endometrium was classified as receptive or non-receptive according to the ERA assessment, and pET was done at the time determined by ERA in the ERA group. Subjects in the control group did not receive ERA and underwent blastocyst transfer normally.
Main results and the role of chance: The demographic and clinical characteristics were comparable between the ERA and control groups ($P > 0.05$). The ERA test identified 10.45% of samples as receptive and 89.55% of samples as non-receptive in the ERA group, with 70.15% of samples presenting a pre-receptive profile. We observed higher cumulative pregnancy (74.63% vs. 64.10%) and cumulative implantation rate (47.32% vs. 21.68%) rates, and a lower biochemical pregnancy rate (18.00% vs. 34.00%) in the ERA group when compared to the control group ($P < 0.05$). Additionally, we found higher pregnancy (67.16% vs. 39.74%) and implantation (46.54% vs. 16.94%) rates as well as a lower biochemical pregnancy rate (17.78% vs. 45.16%) after the first ERA test in the ERA group when compared to the control group ($P < 0.01$).
Limitations, reasons for caution: First, this is a retrospective analysis, which is relatively more biased than prospective clinical trials. Second, the study sample is considerably small. Third, only 10.45% of the subjects were identified as presenting a receptive profile, which limits the comparisons of clinical outcomes between patients with receptive and non-receptive endometria.
Wider implications of the findings: This study demonstrates that the ERA test helps to determine the optimal timing for embryo transfer, improve pregnancy and implantation rates in patients with RIF, and guides the clinical application of the ERA test.
Trial registration number: approval No. 2020-018

P-314 Serum Stem Cell Factor as a predictor of top quality blasotcysts formation in women with endometriosis

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Study question: To evaluate the correlation between the serum level of stem cell factor (s-SCF) during the stimulation and results of embryo culture.
Summary answer: The serum SCF concentration at the stimulation stage may be a potential predictor of IVF outcome in endometriosis patients.
What is known already: Stem cell factor (SCF) is a pleiotropic cytokine that affects the target cells via the c-kit receptor, a tyrosine kinase receptor. Recent evidence indicates that SCF and c-kit may play a role in regulation and growth of ovarian follicular function. It is unclear whether endometriosis primarily affects in vitro fertilization outcomes via oocyte quality. SCF is produced during the human follicular phase, immediately before the ovulatory phase, and may play an important role in folliculogenesis and in the mechanism of ovulation. It may reflect a successful stimulation with ample follicle maturation.
Study design, size, duration: This was a prospective case-control study and consisted four group of patients: 10 with endometriosis, 24 PCOs, 20 with normal (AMH 1.2-4.0 ng/ml) and 11 with lower (AMH<1.2 ng/ml) ovary reserve who were undergoing IVF treatment with the assessment of serum SCF concentration between August 2019 and March 2020 at INVICTA Fertility Centre, Poland. The age of the patients ranged from 22 to 42 years (median 34 years).
Participants/materials, setting, methods: s-SCF was measured in duplicate by enzyme-linked immunosorbent assay (ELISA) kit in 195 serum samples collected during ovarian stimulation on days 1 and 8 and on the day of oocyte retrieval. We analysed correlation between s-SCF level and formation of top quality (TQ) blastocysts on day 5 formation in the study groups.
Main results and the role of chance: We have compared mean level of s-SCF within each group dividing the patients into two subgroups – those with at least one TQ blastocyst (TQ) on day 5 vs. those with no TQ blastocysts (no-TQ). There were no significant differences in mean s-SCF level on day 1 of stimulation between no-TQ and TQ patients in PCOs, normal and lower ovary reserve groups (41.1 pg/ml vs. 40.9 pg/ml; 34.8 pg/ml vs. 38.9 pg/ml and 32.3pg/ml vs. 28.7 pg/ml respectively). The mean level of s-SCF in endometriosis patients was higher in case of no-TQ compared to the TQ subgroup and were 41.1 pg/ml and 29.1 pg/ml respectively. Also no significant differences were also observed in the mean level of s-SCF in the no-TQ and TQ subgroups on the 8 day of stimulation and pick-up in PCOs, normal and lower ovary reserve patients. However, again in the case of endometriosis patients, the mean level of s-SCF was significantly lower on the 8 day of stimulation (28.1 pg/ml vs. 49.1 pg/ml; $p<0.05$) and pick-up day (33.4 pg/ml vs. 50.4 pg/ml; $p<0.005$) in samples from patients who had at least one TQ blastocysts on day 5 of culture.
Limitations, reasons for caution: More data are required to confirm the corelation of s-SCF level and presence of top quality blastocysts in patients with endometriosis.
Wider implications of the findings: Our study suggests that the level of serum SCF during ovarian stimulation in patients with endometriosis of less 30 pg/ml may potentially be a predictor for the chance of obtaining at least one top quality blastocyst on day 5 and thus a chance to successful treatment.
Trial registration number: not applicable

P-315 Ultrasound diagnosis of adenomyosis: impact on pregnancy rate in ivf cycles with donated oocytes

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Study question: The aim was to evaluate in patients who underwent embryo transfer (ET) in an oocyte donation cycle, the impact of adenomyosis, diagnosed by transvaginal sonographic (TVS), on the implantation rate.
Summary answer: We observed a slightly higher miscarriage rate in the first trimester in patients with adenomyosis in particular in the diffuse type.
What is known already: What we know from literature is that there are pro studies such as Costello and Vercellini's which show a reduced pregnancy rate and birth rate, and cons studies which find no effects at all of adenomyosis on IVF treatments. However, both show an increased risk of miscarriage and obstetric complications
Study design, size, duration: This prospective observational study involved a total of 72 patients: 33 with adenomyosis and 39 without adenomyosis from June 2019 to December 2020. All had a workup which included history, pelvic exam and 2/3D TVS scan which was saved as images, videoclips and volumes and stored. The off line evaluation was performed blind to IVF indication and outcomes by expert sonographer, who assessed the presence or absence of TVS signs of adenomyosis.
Participants/materials, setting, methods: All the patients aged ≤ 45 years old undergoing, for several personal problems, their first oocyte donation at IVI center Rome.Patients were divided into 2 groups according to findings on a baseline pre-treatment TVS: patients with and without adenomyosis. In the patients with adenomyosis, the disease was further classified according to type (diffuse,focal), localization (inner and outer myometrium) and extension inside the uterus (mild, moderate, severe) and correlated to pregnancy rate and outcome
Main results and the role of chance: A total of 72 patients were included in this study: 33 with adenomyosis and 39 without adenomyosis. The presence, type and degree of adenomyosis doesn't show a correlation to embryo implantation rate (64.1% in the control group vs 63.6% in adenomyosis group). However we found an increased risk of early miscarriage in the patients with adenomyosis (12% in the control group vs 23.8% in adenomyosis group). Women with adenomyosis that infiltrated only the external myometrium showed a lower pregnancy rate (40%) compared to those who had the involvement of only the inner myometrium (77,7%). The presence of ultrasound findings of focal disease was associated with a lower pregnancy rate (53,3%) compared to the diffuse disease (72,2%); We observed a slightly higher miscarriage rate in the first trimester in patients with adenomyosis in particular in the diffuse type .The presence, type and degree of adenomyosis doesn't show a correlation to embryo implantation rate.
Limitations, reasons for caution: Most of the patients included in our study has an age > 40. This could determine an increased number of high-risk pregnancies.
Wider implications of the findings: Results of this study may be used to evaluate the impact of different medical or surgical treatment in women with adenomyosis undergoing IVF.
Trial registration number: not applicable

P-316 A rat model of endometrioma to study endometriosis-related infertility

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Study question: Underlying mechanisms and specific treatment for endometriosis-related infertility are still unclear and lacking. Is there any animal model suitable for the study?
Summary answer: Endometium ligation to ovary fat pad in rats is a more appropriate and successful but less detrimental model of endometrioma for infertility pathology and outcomes.
What is known already: Nonhuman primates (NHP) have been the most representative animal model for endometriosis since they menstruate in a cyclic pattern and develop endometriosis spontaneously as in human. However, the incidence of spontaneous endometriosis in NHP is low and due to ethical concerns and high cost, the application of NHP for endometriosis study is restricted. Rodents (i.e. rats, mice, rabbits) have been used to analyze the pathophysiology of endometriosis. Because rodents do not develop endometriosis spontaneously, it has to be induced surgically. Ovarian endometriosis is the most common type, but previous experimental models were mostly either subcutaneous, peritoneal wall or mesenteric endometriosis.
Study design, size, duration: An animal study to compare different methods of endometrium transplantation to the ovary as potential animal model of endometrioma to study the endometriosis-related infertility. Compared with NHP and other rodents, rats were chosen due to easy access, low cost, good ovary size, short estrus and reproductive cycle and similar endocrine pattern to human beings. For each transplantation method, at least 5 animals were included and followed up in different time points for comparisons.

Participants/materials, setting, methods: To establish ovarian endometriosis or endometrioma model, uterine tissues were collected from donor rats and then transplanted to recipient rats by either adhesion to ovary, ligation to ovary fat pad, or injection underneath tunica albuginea. Vasculature and histology of engrafts, follicle count and morphology of ovary, receptivity markers of endometrium, immune response and inflammatory cytokines of peritoneal cavity and hormonal changes were assessed after 4 days of transplantation. Implantation rates after conception will be examined.

Main results and the role of chance: Compared with other transplantation methods, only ligation of endometrium fragments to ovary fat pad resulted in endometrioma-like lesion in the ovaries. Compare with sham control ovaries, formation of new vessels from surrounding ovarian vessels to the endometriotic engraft assessed by Cellvizio LAB in vivo imaging was identified, indicating active angiogenesis. Morphology and histology of endometriotic cyst were confirmed in the lesion by H&E staining, suggesting functional endometrium. Stroma markers CD140b, CD106 lkand epithelium markers keratin 17/19 and EpCam were found in the ovary cortex, implying integration of endometrium tissue to the ovarian tissues. Decreased AMH expression and antral follicle count in the ovaries suggested defective follicle development. Reduced receptivity markers HOXA10, LIF and αvβ3 integrin expression in uterine endometrium and implantation rate after nature mating or embryo transfer indicated potential impaired endometrial receptivity. Peripheral and peritoneal levels of COX2 and IL-8 were elevated, suggesting inflammatory response. However, estrogen and progesterone levels were not significant different from baseline and other transplantation methods.

Limitations, reasons for caution: This was an animal model, it might not totally reflect the exact pathological changes of endometrioma in human. Current method was a single and short-term transplantation model, monthly endometrial cells engraftment and slow growing lesions without sufficient estrogen supply might limit the establishment of endometrium-like lesions. Further testings are needed.

Wider implications of the findings: Establishment of animal models for endometrioma is vital important to investigate the pathophysiology, underlying mechanism and potential therapy targets for endometriosis-related infertility. Currently such model is still lacking. An appropriate, adequate and effective but less detrimental model of endometrioma can accelerate the scientific research and clinical application in near future.

Trial registration number: N/A

P-317 Uterine cavity in patients with repeated implantation failure (RIF) and before the first IVFprogram

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Study question: What is the frequency of uterine pathology and histological analysis of chronic endometritis in the RIF group and in patients before the first IVF program?

Summary answer: We report a lower incidence of chronic endometritis in the group of patients just preparing for the first IVF program and higher effectiveness

What is known already: Chronic endometritis (CE) is one of the reasons for implantation failures in IVF cycles. The presence of chronic inflammation in the endometrium changes its susceptibility, resulting in failed implantations and loss of pregnancy. CE diagnostics is rather complicated. Visual examination during hysteroscopy allows one to suspect CE in 20-70%, routine histology - in 10-20%. The new gold standard is immunohistochemistry (IHC).

Study design, size, duration: A continuous study of 2003 hysteroscopies was carried out, conducted in 1944 patients from May 2018 to January 2020. All patients were divided into 2 groups: 1 - 650 patients preparing for IVF for the first time; 2 - 1294 with repeated implantation failures.

Participants/materials, setting, methods: All included patients had diagnostic office hysteroscopy (OH) with biopsy and IHC CD 138, CD 20, and CD 8. We compared the frequency of CE detection by visual examination during hysteroscopy, routine histology and IHC of the two groups, as well as the effectiveness of 1 course of antibiotic therapy with CE.

Main results and the role of chance: In group 1 of women preparing for IVF for the first time, during visual examination by hysteroscopy, CE was detected in 17% (110/650), in group 2 RIF - in 27% (349/1294), p <0.001. Routine histology also more often detected CE in the RIF group, 42% (543/1294) compared with group 1 - 23% (149/650), p <0.001. IHC markers of CE were detected in 81.8% (1058/1294) of samples from RIP group than in group 1 - 71.4% (464/650), p <0.001. At the same time, IHC mild CE was more common in group 1 in 320/650 (49.2%), p <0.001. In the second group, mild, moderate and severe CE occurred with the same frequency (33.2%, 36.5%, 30.3%, respectively). The frequency of CE detection by IHC in the groups after 1 course of antibiotic therapy with fluoroquinolones was 31.2% (145/464) and 43.2% (457/1058), respectively, p <0.001. The pregnancy rate (PR) also differed and was the lowest in the RIF group: in group 1 after treatment with CE PR = 42.7% (198/464), in group 2 after treatment with CE PR = 27.2% (288 / 1058), p <0.05.

Limitations, reasons for caution: The limitations are related to study design. More research is also needed.

Wider implications of the findings: The question of overdiagnosis of CE using IHC remains open. Therefore, well-designed prospective studies or RCTs should be conducted to clarify possible correlations between ChE and poor reproductive outcomes and the effectiveness of endometrial interventions and treatments.

Trial registration number: not avaliable

P-318 Short (seven days) versus conventional (fourteen days) estrogen priming in an artificial frozen embryo transfer cycle: a randomised controlled trial

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Study question: What is the impact of seven days versus fourteen days' estrogen (E2) priming on the clinical outcome of frozen-embryo-transfer in artificially prepared endometrium (FET-HRT) cycles?

Summary answer: No significant difference in clinical/ongoing pregnancy rate was observed when comparing 7 versus 14 days of estrogen priming before starting progesterone (P) supplementation.

What is known already: One (effective) method for endometrial preparation prior to frozen embryo transfer is hormone replacement therapy (HRT), a sequential regimen with E2 and P, which aims to mimic the endocrine exposure of the endometrium in a physiological cycle. The average duration of E2 supplementation is generally 12-14 days, however, this protocol has been arbitrarily chosen whereas, the optimal duration of E2 implementation remains unknown.

Study design, size, duration: This is a single-center, randomized, controlled, open-label pilot study. All FET-HRT cycles were performed in a tertiary centre between October 2018 and December 2020. Overall, 150 patients were randomized of whom 132 were included in the analysis after screening failure and drop-out.

Participants/materials, setting, methods: The included patients were randomized into one of 2 groups; group A (7 days of E2 prior to P supplementation) and group B (14 days of E2 prior to P supplementation). Both groups received blastocyst stage embryos for transfer on the 6th day of vaginal P administration. Pregnancy was assessed by an hCG blood test 12 days after FET and clinical pregnancy was confirmed by transvaginal ultrasound at 7 weeks of gestation.

Main results and the role of chance: Following the exclusion of drop-outs and screening failures, 132 patients were finally included both in group A (69 patients) or group B (63 patients). Demographic characteristics for both groups

were comparable. The positive pregnancy rate was 46.4% and 53.9%, (p 0.462) for group A and group B, respectively. With regard to the clinical pregnancy rate at 7 weeks, no statistically significant difference was observed (36.2% vs 36.5% for group A and group B, respectively, p=0.499). The secondary outcomes of the study (biochemical pregnancy, miscarriage and live birth rate) were also comparable between the two arms for both PP and ITT analysis. Multivariable logistic regression showed that the HRT scheme is not associated with pregnancy rate, however, the P value on the day of ET is significantly associated with the pregnancy outcome.

Limitations, reasons for caution: This study was designed as a proof of principle trial with a limited study population and therefore underpowered to determine the superiority of one intervention over another. Instead, the purpose of the present study was to explore trends in outcome differences and to allow us to safely design larger RCTs.

Wider implications of the findings: The results of this study give the confidence to perform larger-scale RCTs to confirm whether a FET-HRT can be performed safely in a shorter time frame, thus, reducing the TTP, while maintaining comparable pregnancy and live birth rates.

Trial registration number: NCT03930706

P-319 Measuring intraobserver and intermethod reliability of endometriotic cyst volumes: a comparison between MRI and 3D transvaginal ultrasound

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Study question: Are three-dimensional imaging techniques, MRI and three-dimensional transvaginal ultrasound reliable for the volume measurement of endometriotic cysts?

Summary answer: MRI and XI VOCAL 3D-transvaginal ultrasound both provide a very good intraobserver reliability. The imaging techniques are however not advised to be used interchangeably.

What is known already: Two-dimensional transvaginal ultrasound (2D-TVUS) and MRI are commonly used in endometriosis care. However, three-dimensional ultrasound (3D-US) has gained more attention in recent years. The use of 3D-US, more specifically VOCAL and XI VOCAL software has proven to be a reliable tool in the measurement of volumes such as splenic volumes and uterine niche volumes. Up to date, 3D-TVUS has not been evaluated for volume measurements in endometrioma.

Study design, size, duration: A prospective case-control study was performed in an academic endometriosis centre. In total, 23 endometriosis cysts from 16 patients were included.

Participants/materials, setting, methods: Women diagnosed with endometriosis through laparoscopy with histological confirmation presenting with uni- or bilateral endometrioma on TVUS were included in this study. All women had a regular menstrual cycle (28 days ± 3 days). Women were seen for examinations at two time points during one menstrual cycle: on cycle day 2-4 (T0) and cycle day 20-22 (T1). At both time points a 2D and 3D TVUS and an MRI at 1,5T were performed.

Main results and the role of chance: The intraclass correlation for intraobserver reliability is good to very good for all three techniques ranging from the lowest value of 0.953 to the highest of 1.000. MRI has the most narrow limits of agreement (-3.93 to 4.53), followed by XI VOCAL (-5.16 – 5.65) while VOCAL has the widest limits of agreement (-10.22 to 11.39). Intraclass correlations are poor in the comparison of XI VOCAL to MRI, moderate between VOCAL and XI VOCAL, and good for the comparison between VOCAL and MRI. The limits of agreement are widest for XI VOCAL versus MRI (-36.96 -10.54). Similar limits of agreement are found between VOCAL versus XI VOCAL (-7.80 -23.40) as between VOCAL versus MRI (-18.27-8.96).

Limitations, reasons for caution: No absolute volume measurements were obtained during subsequent surgery to compare the imaging data to. This makes it more difficult to determine an acceptable error margin for the 3D imaging techniques used.

Wider implications of the findings: Being able to determine (small) volumetric changes in endometrioma can give more insight into the development and circumstances under which endometrioma grow or decrease in size. For reliable and accurate follow up of endometrioma a single measuring technique must be used as three-dimensional imaging techniques are not interchangeable.

Trial registration number: Trial NL2106 (NTR2223)

P-320 Endometrial flushing to improve fertility outcomes: a systematic review and meta-analysis

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Study question: Does flushing the endometrial cavity improve fertility outcomes in couples with infertility and what are the preferred flushing mediums?

Summary answer: Flushing the endometrial cavity appears to improve live birth rate, implantation rate and clinical pregnancy rate. There was no difference in miscarriage or complication rate.

What is known already: Live birth rate was improved in cleavage-stage transfer when the uterus was flushed with human chorionic gonadotropin (hCG) at a dose >500 IU in a Cochrane review (Craciunas L et al 2018). Chemical and clinical pregnancy rates and implantation rate may be improved by endometrial flushing with platelet-rich plasma (PRP) in a meta-analysis by Maleki-Hajiagha A et al 2020. Flushing the cavity with oil-based contrast at hysterosalpingography (HSG) improved pregnancy rates when compared with water-based contrast in a systematic review by Fang F et al 2018.

Study design, size, duration: Our study was a systematic review and meta-analysis. We searched MEDLINE (1946 to December 2020), Embase (1980 to December 2020), Cumulative Index to Nursing and Allied Health Literature (CINAHL) (1961 to 9 December 2020) PsychINFO (1806 to December 2020). We screened the titles of search results to identify studies for inclusion and then screened abstracts and full texts to ensure trials met search criteria.

Participants/materials, setting, methods: We included in this review all randomised controlled trials (RCTs), quasi-experimental or cohort studies evaluating intrauterine instillation or flushing with any medium, irrespective of timing of intervention and irrespective of language or country of origin. We performed the meta-analyses using a random-effects model and performed subgroup and sensitivity analysis. Two authors independently extracted the data. We calculated the risk ratio or odds ratio with 95% confidence intervals based on intention-to-treat or available data analysis.

Main results and the role of chance: We included 36 RCTs and 12 non-randomised trials including 12230 participants. The sample size varied from 15 to 1186. We included 11 comparisons. Primary outcomes were healthy baby rate and live birth rate. Healthy baby rate was not reported in any of the trials. Live birth rate favoured oil soluble contrast medium (at HSG) when compared to water based contrast medium (OR 0.64, 95% CI 0.52 to 0.78, 2 RCTs and 1 cohort study, 2050 participants, I2 = 86%) or no intervention (OR 3.53, 95% CI 1.64 to 7.60, 2 RCTs, 192 participants, I2 = 0%). With regards to our secondary outcomes: CSF instillation, HCG instillation, PRP instillation and oil-soluble contrast medium at HSG (over water-soluble contrast medium) were favoured for implantation rate and clinical pregnancy rate. There was no statistically significant difference in miscarriage or complication rate for any of the comparisons. All trials were at an overall high risk of bias when assessed on the following areas: sequence generation, allocation concealment, blinding of participants, personnel, and outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias.

Limitations, reasons for caution: Only 36 studies included were RCTs. Twelve of the RCTs had post randomisation drop outs. There was a lack of detail on the instillation medium for several of the included studies. There was no placebo used in many of the control groups. All meta-analyses had high levels of heterogeneity.

Wider implications of the findings: Our findings are in keeping with the literature suggesting a possible improvement in live birth rate following flushing the endometrium with oil soluble contrast medium when compared with a

control group. Mechanistic studies are now required to understand how oil-based medium improves fertility.
Trial registration number: NA

P-321 The impact of endometrioma and ovarian cystectomy in patients with major indications for IVF/ICSI with endometriosis

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Study question: Does presence of endometrioma has worse IVF/ICSI outcome than endometriosis per se? What about the impact of cystectomy of endometrioma on IVF/ICSI outcomes?
Summary answer: IVF/ICSI outcome of patients with endometrioma is comparable than with endometriosis. Cystectomy for endometrioma did not alter IVF/ICSI outcomes if ovarian reserve is comparable.
What is known already: Previous studies revealed women with endometrioma undergoing IVF/ICSI had similar reproductive outcomes compared with those without. Most of the comparisons are between women with endometrioma and women without endometriosis. However, endometrioma per se, different from endometriosis may have specific impact on IVF/ICSI outcomes. There is now molecular, histological and morphological evidence to suggest endometrioma is detrimental to the ovaries. Studies comparing IVF/ICSI outcomes between women with endometrioma and women with endometriosis are few.

Cystectomy of endometrioma may worse ovarian reserve, and subsequently adversely affect IVF/ICSI outcomes. But there are possible complications associated with the persistence of endometrioma during IVF/ICSI.
Study design, size, duration: Retrospective analysis of 2153 IVF/ICSI cases during Jan/01/2014 to Dec/31/2018 in VGHTC. We included women who received ART due to endometriosis(n=208). Exclusion criteria including patients >40 years-old, simulation day < 5 days, severe male factor, uterine factor (including adenomyosis) and immunological factors. Patients whose embryos were not completely transferred back or who received embryo transfer from different OPU cycles are excluded. We followed up these patients till 2020/6. The primary outcome is cumulative LBR
Participants/materials, setting, methods: For first analysis, we divided 208 cases to patients with endometrioma during IVF/ICSI(n=89), and patients only diagnosed of endometriosis (n=119). Second analysis on the effect of cystectomy of endometrioma on IVF/ICSI outcomes. Patients with endometrioma (n=89) during IVF/ICSI were further divided to patients with primary endometrioma (n=70) and patients with recurrent endometrioma (n=19, ever received cystectomy for endometrioma). Another group is patients without endometrioma during IVF/ICSI, but ever received cystectomy before (n=40)
Main results and the role of chance: For the first analysis, age, BMI and AMH were comparable in endometrioma (n=89) and endometriosis group(n=119). The usage gonadotropin dose was significantly higher in the endometrioma group (FSH 3619IU vs 3471IU, p=0.001. LH 1224 IU vs 941 IU, p=0.009). The Blastocyst formation rate is lower in the endometrioma group (49.4% vs. 57.7% p=0.005). The OPU number, LBR and cumulative LBR were comparable in both groups (10.3 vs 12.4 p=0.131, 33.3% vs 37%, p=0.687, 49.4% vs 60.5%, endometrioma vs endometriosis). For the second analysis, when comparing cystectomy before IVF/ICSI group with primary endometrioma group, cystectomy group were younger (32.8 vs 34.8 p=0.006). AMH level were comparable. The BC formation rate was significantly higher in the cystectomy group (61.5% vs 50.4% 0= 0.007). The LBR and cumulative LBR were comparable in both groups (43.5% vs 28.1%, 60% vs 48% in cystectomy vs primary endometrioma group). As for the recurrent endometrioma group, the age and AMH level were comparable with cystectomy group, but the usage gonadotropin dose was significantly higher than other two groups. The BC formation rate was also lower than cystectomy group (47.8% vs 61.5% p=0.042). The LBR and cumulative LBR were comparable with other two groups (55.6%, 57.9%).
Limitations, reasons for caution: This is a retrospective study, and the sample size is limit. We did not analysis the size of endometrioma nor the unilateral or bilateral endometrioma.
Wider implications of the findings: Cystectomy for endometrioma must be carefully selected since it did not alter IVF/ICSI outcome only if the ovarian reserve is not affected. Recurrent endometriomas do not have a worse impact on IVF/ICSI outcome than primary endometrioma. If there is recurrent endometrioma, IVF/ICSI may be the first priority.
Trial registration number: not applicable

P-322 Addressing progesterone and cAMP signalling pathways for decidualization induction of endometrial stromal cells of patients with endometriosis

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Study question: Which compounds/compound combinations are most effective in decidualization induction of endometrial stromal cells (ESCs) of patients with and without endometriosis?
Summary answer: Combination of compounds addressing different steps in the signalling cascade of decidualization induce decidualization more effectively than application of the individual compounds alone.
What is known already: Decidualization is the monthly recurring differentiation process of the ESCs in preparation for embryo implantation in human. Undifferentiated ESCs reveal an increased potential to proliferate and invade after retrograde menstruation. This may lead to the formation of ectopic lesions and the manifestation of the chronic gynaecological disease of endometriosis due to an impairment of the decidualization process.
Study design, size, duration: Compounds and compound combinations addressing the progesterone receptor- or the cAMP-mediated pathway were evaluated with regard to their own and their synergistic potential to induce decidualization of ESCs from women with (n=10) and without (n=10) endometriosis during a 6-day treatment.
Participants/materials, setting, methods: Human primary ESCs were isolated via enzymatic-mechanic digestion from eutopic endometrium from women with and without endometriosis and treated for 6 days in vitro with different progestins (progesterone, medoxyprogesterone acetate (MPA)), 8-Br-cAMP, forskolin, or phosphodiesterase (PDE)-inhibitor (Rolipram) alone or in combination. The degree of decidualization induction was quantified by morphological, biochemical (prolactin) and molecular (HAND2, FOXO1) parameters by means of ELISA, flow cytometric analysis, Realtime PCR and Western blot analysis.
Main results and the role of chance: After 6 days of treatment, decidualization was induced by forskolin as well as by 8-Br-cAMP whereas progestins or PDE alone hardly induced prolactin secretion by ESCs as a marker of decidualization. A change of morphology from undifferentiated fibroblast-like cells to rounded cells could be observed in parallel with the secretion of prolactin. Forskolin and 8-Br-cAMP-induced decidualization was significantly enhanced by MPA but not by progesterone. These effects were similar in ESCs from women with and without endometriosis. Moreover, forskolin-induced decidualization was significantly enhanced by simultaneous application of PDE. Interestingly, this effect was higher in cells of patients with endometriosis. An induction of decidualization in ESCs was associated with a parallel increase of the process-associated transcription factors HAND2 and FOXO1. This rise of transcription was markedly increased in combination with MPA but not with progesterone.
Limitations, reasons for caution: Endometrial tissue was obtained from women undergoing infertility treatment and thus may differ from the endometrium of fertile women. Results obtained from primary cells in vitro may not cover the in vivo situation in all respects.
Wider implications of the findings: The results of this study provide baseline data for the development of a possible therapeutical approach to induce decidualization as a treatment option for endometriosis. Further research is required to determine the effectiveness of the in vitro tested compound combinations in an in vivo model.
Trial registration number: not applicable

P-323 Ovarian response to stimulation according to endometrioma size, in women with deep infiltrating endometriosis – A comparative study

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Study question: Does endometrioma (OMA) size affect the number of oocytes retrieved after ovarian stimulation (OS) in women with deep infiltrating endometriosis (DIE)? Summary answer: No significant difference in the number of oocytes retrieved was observed according to the endometrioma size.
What is known already: Ovarian endometriosis lesions (OMA) *per se* and above all, the surgical excision, appears to result in a risk of alteration of the ovarian reserve. In vitro fertilization (IVF) is a validated therapeutic option to treat infertility related to endometriosis. Nevertheless, it has been described that the presence of OMA could have a detrimental impact on ovarian responsiveness to hyperstimulation involving mechanisms still unclear. Some recent studies suggest that the size of the OMA may be relevant and that there may be a threshold in cyst diameter above which ovarian responsiveness might be affected. Study design, size, duration: This was an observational study using data prospectively collected in a cohort of infertile women aged between 18 and 43 years presenting OMA associated with DIE lesions, between December 2012 and July 2019. Every patient underwent their first in vitro fecundation or intracytoplasmic sperm injection (IVF/ICSI) cycle. Included women were women with an adequate imaging work up with Transvaginal ultrasound and/or magnetic resonance imaging (TVUS/MRI) performed by senior radiologists before the beginning of the OS.
Participants/materials, setting, methods: One hundred and eighty-two women were included in the study. Women were allocated in 5 groups according to the largest diameter of their ovarian endometriosis lesions: OMA < 2 cm, 2 cm ≤ OMA < 4 cm, 4 cm ≤ OMA < 6 cm, 6 cm ≤ OMA < 8 cm, OMA ≥ 8 cm. The main outcome was the number of oocytes retrieved.
Main results and the role of chance: Mean age of the included women was 32.8 years. 96(52.7%) women had unilateral endometrioma and 86 (47.3%) had bilateral endometriomas. The mean OMA size was 3.63 cm for right ovary and 3.60 cm for left ovary. Considering the largest diameter of OMA retained, the mean size was 4.12 cm. Repartition among groups, according to the size of the largest OMA diameter was: OMA < 2cm group (n = 32); 2 £OMA< 4 cm (n=70); 4 £OMA< 6 cm, (n = 37); 6 £OMA< 8 cm (n = 27); OMA8 cm (n = 16).
Mean number of oocytes retrieved was not significantly different between groups (p=0.635): 8.4±5.7 for OMA< 2 cm, 7.3 ± 5.4 for 2 cm≤OMA<4 cm, 6.6 ± 3.9 for 4 cm≤OMA< 6 cm, 8.6 ± 5.8 for 6 cm ≤OMA<8 cm and 7.1 ± 3.6 for OMA≥8 cm. Mean number of matures oocytes was also comparable between groups (p = 0.674). Clinical pregnancy rate and live birth rate was similar between groups (p= 0.798 and p =0.913). No significant difference was found concerning the number of cancelled cycles between groups (p= 0.703).
Limitations, reasons for caution: For almost half of the included women, endometriosis diagnosis was based on imaging technics, without histological proof of endometriosis. However, it was performed by specialized seniors radiologists.
Wider implications of the findings: Our study suggests that whatever endometrioma size, OS can be benefit for women with endometrioma, even for largest ones, without the requirement of prior treatment to reduce their size
Trial registration number: NA

P-324 Pregnancy outcome after IVF for endometriosis or male infertility: What differs?

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Study question: Are endometriosis women pregnant after IVF at increased risk of preeclampsia or placenta praevia than patients monitored for male infertility?
Summary answer: Patients with endometriosis are at greater risk than patients monitored for male infertility of developing preeclampsia and placenta previa.
What is known already: Endometriosis is a chronic estrogen-dependent disease that affects women of childbearing age which represents 10% of the general population[.The main symptoms found are chronic pelvic pain, infertility, dyspareunia and dysmenorrhea. Numerous publications have highlighted the deleterious effect of endometriosis on pregnancy i.e miscarriage, placental abnormalities, preeclampsia, preterm birth, low gestational weight. This complication may be related to the molecular and cellular abnormalities present in the endometrium of these patients and to the inflammatory state that may lead to abnormal contractility of the uterus at the time of the implantation window and trophoblastic invasion.
Study design, size, duration: This study is a retrospective, non-interventional monocentric cohort study conducted between January 2011 and December 2017 in Institut de Medecine de la Reproduction - Clinique Bouchard in Marseilles, France.
Participants/materials, setting, methods: The outcome of pregnancies obtained after IVF and/or ICSI in patients with endometriosis (n=270) was compared with patients,free of endometriosis,monitored for male infertility (n=366) The statistical study was carried out using GraphPad Version 8 The Student T-test was used to compare means across them. Results were considered significant for $p < 0.05$.
Main results and the role of chance: Patients with endometriosis and monitored during this period were older than those managed for male infertility. (33.59 vs 32.78) (p = 0.04). There was no difference between the two populations regarding BMI (p=0.31) or smoking (p>0.9). The rate of miscarriage observed in the two populations was comparable (25.37 vs. 25.78%) (p>0.9), so was the rate of IUGR (5.81% vs. 2.29%) despite the observed percentages (p>0.9). The rate of premature deliveries did not differ between the two populations (18.37% vs. 14.29%) (p=0.55) neither did the number of children born with a weight <2500g at term (13.68% vs. 12.5%) (p=0.83). Although the rate of gestational diabetes was comparable in both groups (4.11% vs 4.56%), the rate of preeclampsia was higher in the group of patients with endometriosis with a statistically significant difference (4.79% vs 0.79%) (p=0.01). Similarly, the rate of placenta previa was higher in patients with endometriosis (4.11% vs 0.76%) (p=0.02). All pregnancies complicated by placenta previa resulted from J2/J3 embryo transfer. Estradiol levels on the day of induction (2166 pg/ml vs 2452) (p=0.67) and endometrial thickness was not different between patients with placenta praevia or no (10.45 vs 10.51) (p=0.66).
Limitations, reasons for caution: Our study is retrospective which may introduce several biases despite the size of our sample i;e patients with endometriosis are older, adenomyosis was not included in the criteria. In our study we have not found any additional risk related to the type of embryo transferred.
Wider implications of the findings: Patients with endometriosis are at greater risk than patients managed for male infertility of developing preeclampsia and placenta previa. It is advisable to warn patients of this possible complication, to promote e-SET and to set up early monitoring in order to place the appropriate management around these patients.
Trial registration number: NOT APPLICABLE

P-325 The impact of letrozole on endometrial thickness in IVF cycles

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Study question: Does daily administration of letrozole during IVF stimulation affect endometrial thickness ?
Summary answer: Patients treated with letrozole during fresh IVF cycles had a thinner endometrium on the day of trigger compared to patients who did not receive letrozole.
What is known already: Letrozole supplementation is commonly used during fertility preservation for breast cancer patients to reduce peak estrogen levels with no adverse effects on embryo outcomes. Studies in poor responders have found that letrozole use resulted in a shorter duration of stimulation and a lower total dose of gonadotropin, with no detrimental effect on IVF outcomes. In normal responders, studies have shown an increase in blastocysts obtained, but have not yet shown an increase in clinical pregnancy rates. There is concern that

when a fresh embryo transfer is planned letrozole use may negatively affect endometrial thickness and subsequently diminish pregnancy rates.
Study design, size, duration: In a retrospective cohort study between January 2009 and June 2019 at a single academic fertility center, we compared the endometrial thickness in 97 cancer patients who underwent IVF-fertility preservation with daily letrozole use to 158 cancer patients who underwent IVF-fertility preservation without letrozole.
Participants/materials, setting, methods: All women diagnosed with cancer were referred for fertility preservation prior to gonadotoxic treatment exposure and were less than 40 years old at the time of oocyte retrieval. All patients who received letrozole started on day one of stimulation and continued until the day of oocyte retrieval. The primary outcome was endometrial thickness on the day of trigger. The secondary outcomes were number of oocytes retrieved, number of MII retrieved, and maximal estradiol level.
Main results and the role of chance: During the study period, 336 cancer patients underwent fertility preservation. Eighty-one patients were excluded; 50 because they had an intrauterine device or were on long term oral contraceptives and 31 because endometrial thickness was not documented. Of the remaining 255 patients, 86 had breast cancer, 95 had a hematological cancer and 74 had various other cancers. Ninety-seven cancer patients treated with letrozole were compared to 158 cancer patients who did not receive letrozole. Patients who received letrozole were significantly older (34 vs 28yrs, P<0.0001). There were no significant differences in baseline characteristics such as BMI, AFC nor in the total duration for stimulation. Endometrial thickness on the day of trigger was significantly less in letrozole treated patients (8 vs 9mm, P<0.003). There were no significant differences in total number of oocytes retrieved (12.5 vs 11, P=0.126) nor in the number of mature oocytes (8 vs 8, P=0.312). Patients in the letrozole group received a higher total gonadotropin dose (2680IU vs 1980IU, P=0.016). The maximum estradiol level was significantly lower in patients treated with letrozole (1068 vs 3838ml/dl, P=<0.0001). A regression analysis showed that using letrozole during stimulation decreased the endometrial thickness by 0.81mm (CI -1.37 to -0.253, P=0.005).
Limitations, reasons for caution: The retrospective nature of this study could have introduced selection and misinformation bias. We report on cancer patients where all oocytes or embryos were vitrified. Without fresh embryo transfer data, it is unclear if a thinner endometrium due to letrozole will effect the implantation or pregnancy rate.
Wider implications of the findings: As the use of letrozole expands beyond cancer patients and poor responders, it is important to understand the impact on the endometrium. This study shows that letrozole reduces endometrial thickness. However, the effect on endometrial function remains unknown. Further study is needed before letrozole can be used with fresh transfers.
Trial registration number: 2020-6370

P-326 Presence of adenomyosis at MRI in endometriosis women negatively affect live birth chances in IVF cycles

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Study question: What is the impact of adenomyosis and its magnetic resonance imaging (MRI) characteristics on live birth rate (LBR) in endometriosis-affected women undergoing *in-vitro*fertilization (IVF) treatment?
Summary answer: Among women undergoing IVF, the presence of adenomyosis at MRI, and especially T2 high signal-intensity spots within the myometrium have a negative impact on LBR. What is known already: Adenomyosis is a frequent gynecologic disease. With the development of imaging technics for the diagnosis (notably MRI), several adenomyosis phenotypes have been described and fertility issues seem variable according to the lesions characteristics. Moreover, on IVF outcomes, controversial results have been found in studies assessing the impact of adenomyosis. What make the impact-assessment of adenomyosis on fertility issues even more difficult is the frequent association with endometriosis, another known risk factor of infertility. Some data suggested that adenomyosis could worsen IVF prognostics, however there is no clear consensus about the impact of the adenomyosis on IVF outcomes in endometriosis affected-women.
Study design, size, duration: This was an observational study including phenotyped endometriosis patients, aged between 18 to 42 years, who underwent IVF/intra-cytoplasmic sperm injection (ICSI) treatment in a tertiary care center, from June 2015 through July 2018.Only women who had performed a pelvic MRI during the pre-therapeutic ART work-up, were retained for this study. The MRI data were interpreted by radiologists who had expertise in gynaecological MRI.
Participants/materials, setting, methods: A continuous series of 202 endometriosis affected women was included. The women were followed until four ART cycles had been completed, until delivery or until discontinuation of treatment before the completion of four cycles. The primary outcome was the delivery of one or more live infant(s) after up to four IVF/ICSI cycles. Patients and MRI characteristics were compared between women who gave a live birth and those without live birth.
Main results and the role of chance: The mean age of the included population was 32.5 ±3.7 years. 90.1% (182/202) had deep infiltrating endometriosis whereas only 5.4% (11/202) and 4.5% (9/202) had respectively isolated ovarian endometriosis (OMA) and superficial peritoneal endometriosis (SUP). The presence of adenomyosis (internal and/or external lesions) was found in 71.8% (145/202) of included women. The cumulative live birth rate was 57.4% (116/202). Women that gave birth ('live birth +') were significantly younger, (33.3±4.1 vs 32.0±3.3 p = 0.026) and had significant better ovarian reserve parameters (AMH, AFC). The presence of adenomyosis (internal and/or external lesions) (76/116 (65.5%) versus 69/86 (80.2%), p = 0.022) and the presence of T2 high-signal intensity myometrial spots (27/116 (23.3%) and 37/86 (43.0%), p = 0.003) were significantly less frequently found in the group of women 'Live birth +'. After multivariate analysis, the presence of adenomyosis (OR: 0.48 95% CI (0.29-0.99) p = 0.048) and the presence of T2 high-signal intensity myometrial spots (OR: 0.43 95% CI (0.22-0.86) p = 0.018) were independently found to be associated with a decrease in cumulative chances of live birth.
Limitations, reasons for caution: The inclusion of patients from our referral center could constitute a possible selection bias, as those women may have suffered from particularly severe forms of adenomyosis ± endometriosis.
Wider implications of the findings: In women presenting endometriosis, the practitioner should perform an appropriate imaging work-up searching for adenomyosis, to identify prognostic factors and to plan the strategy of patient management in the setting of ART.
Trial registration number: NA

P-327 Patients' perspectives on how to improve the management of endometriosis in France: The ComPaRe-Endometriosis cohort

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Study question: How should endometriosis management be improved from the patient's point of view?
Summary answer: One thousand endometriosis patients proposed 2,587 ideas to improve the management of endometriosis that reflect three main themes: diagnosis, care, and information on the disease.
What is known already: Endometriosis is a gynecologic condition affecting 10% of reproductive-age women. The disease causes severe pelvic pain and has a dramatic impact on women's quality of life. A mean delay of 7 years was described between onset of symptoms and diagnosis. There is an urgent need to reduce this delay and to rethink endometriosis care in order to adopt a more comprehensive and patient-centered approach, as women are often dissatisfied with the care they receive.
Study design, size, duration: This study was carried out in a random sample of endometriosis patients participating in ComPaRe (Community of Patients for Research), a prospective e-cohort of adult chronic disease patients who will be followed-up for 10 years. Participants complete monthly online questionnaires about their life with their disease(s). Recruitment began in January 2017 and is

still ongoing, with currently 44,000 participants, including 10,000 endometriosis patients in the ComPaRe-Endometriosis sub-cohort.
Participants/materials, setting, methods: We selected a random sample of 1,000 participants in ComPaRe-Endometriosis, forming 3 equal groups of age (<25, 25-45, >45 years old) and education (<12, 12-14, >14 years). We conducted a qualitative study to gather their ideas for improving the management of their disease. Participants were asked: "If you had a magic wand, what would you change in your health care?". One interviewer and two patients independently extracted ideas from the open-ended responses using thematic analysis.
Main results and the role of chance: Patients proposed a total of 2,587 ideas to improve the management of endometriosis, which we classified in three main themes: diagnosis, care, and information on the disease. To improve diagnosis, women proposed 724 ideas classified into 11 areas of improvement, including training of health professionals, taking symptoms seriously, improving the diagnosis process, and recognition of the disease by clinicians. To improve care, patients gave 1,677 ideas classified into 71 areas of improvement. For example, they asked for a better pain management, more listening from caregivers, the reimbursement of care or medical treatments, help in accessing clinicians that are expert in endometriosis, and reduced waiting times for medical appointments and exams. Finally, to improve information on the disease, participants suggested 186 ideas classified into 5 areas of improvement, covering more explanation about the disease, public recognition of endometriosis and general awareness, and more research and more explanation of research results.
Limitations, reasons for caution: The results were reviewed by three people in order to reduce the margin of interpretation in the analysis of this open-ended question, but some subjectivity remains. Generalizability may be difficult because the results are linked to the specificities of the French model of care.
Wider implications of the findings: Through the many ideas proposed by patients, we identified a total of 87 areas for improvement in endometriosis diagnosis, care, and information. These results reflect patients' expectations in terms of management of their disease and will be useful to design a better global care for endometriosis from the patients' perspective.
Trial registration number: Not applicable

P-328 Dienogest significantly decreases the size of the cyst and alters Anti-Müllerian Hormone concentration in patients with endometrioma

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Study question: Does utilization of dienogest make any impact on the size of cyst and Anti-Müllerian Hormone (AMH) concentration in patients with endometrioma throughout 12-months?
Summary answer: Although dienogest makes a gradual reduction in the size of endometrioma cyst throughout 12-months, a significant drop in AMH serum concentration was also noticed.
What is known already: According to recent studies, pre-operative serum AMH levels might be illusively increased with parallel to the size of endometrioma which will be a misleading factor while deciding to operate the patient via cystectomy. Although dienogest is one of the medical options that might be commenced in patients with endometrioma cyst, there is limited data about its effect on the size of the endometrioma and hence serum AMH concentration throughout 12 months of follow up.
Study design, size, duration: The current observational cohort study was conducted among patients with endometrioma those treated with dienogest from January 2017 to January 2020. The primary outcome was alteration in diameter of endometrioma cyst at 6th and 12th months of treatment. Secondary outcome was alteration in serum AMH concentration in the same period. Of 104 patients treated with dienogest, 44 patients were excluded due to being treated with any type of surgical intervention during follow up period.
Participants/materials, setting, methods: A total of 60 patients were recruited for the final analysis. Of them, primary symptom was dysmenorrhea, chronic pelvic pain and menstrual irregularity in 16 (26.7%), 25 (41.7%) and 8 (13.3%) patients, respectively. Eighteen patients (30%) were asymptomatic. As 21 patients had bi-lateral endometrioma, size of the leading cyst was considered to be analyzed for the primary outcome measure. Paired-t test was used for comparison of numerical values and $p \leq 0.05$ was taken as statistical significance.
Main results and the role of chance: The mean age was 31.5±8.0 years. In the time point when dienogest was started, the mean size of the endometrioma was 46.3±17.4 mm. The mean serum AMH concentration was 3.6±2.4 ng/ml. After 6 months of treatment, the mean size of the endometrioma decreased to 38.6±14.0 mm which corresponds to a mean difference of 7.8 mm (95% CI: 3.0 to 12.6; p: 0.003). The respective figure for AMH was 3.3±2.7 ng/ml which corresponds to a mean difference of 0.3 ng/ml (95% CI: -0.2 to 0.8; p: 0.23) at 6 months. After 12 months of treatment, the mean size of the endometrioma was 37.5±15.7 mm which corresponds to a mean difference of 8.9 mm (95% CI: 2.9 to 14.9; p: 0.005) at the end of 12 months. The respective figure for AMH was 2.7±1.9 ng/ml which corresponds to a mean difference of 0.9 ng/ml (95% CI: 0.1 to 1.7; p: 0.045) at the end of 12 months. The mean diameter of endometrioma and AMH concentration did not differ throughout the time period between 6th and 12th months of the treatment.
Limitations, reasons for caution: Although herein we present the largest data that depicts the alteration of endometrioma cyst and AMH concentration with the application of dienogest, the lack of control group is a limitation that avoids to perform any comparison.
Wider implications of the findings: A shrinkage after commencement of treatment suggest that dienogest might present improvement in patients with endometrioma with respect to radiological findings, but further studies are required whether a decline in AMH concentration after 12 months refers to a genuine decrease in ovarian reserve or resolution of misleading high pre-treatment levels.
Trial registration number: not available

P-329 Müllerian anomalies and embryo implantation in oocyte donation

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Study question: Do patients with Mullerian anomalies (MA) who receive donated oocytes have different embryo implantation rate than patients with normal uterus?
Summary answer: In oocyte donation, patients with MA had lower implantation rate than patients with normal uterus.
What is known already: MA are associated with infertility and miscarriage but the mechanisms to explain this relation are not known. Some studies describe both oocyte and/or uterine factor. All studies describing the outcome in patients with MA, so far, are with own oocytes but none in oocyte donation.
Study design, size, duration: A multicentre restrospective cohort study from January 2000 to December 2019. Patients receiving donated oocytes were divided between those with MA (n= 473) according ESHRE classification and other group with normal uterus (n= 57 869). The primary outcome was implantation rate at fresh embryo transfer. Secondary aims were biochemical pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate, miscarriage rate and live pregnancy rate.
Participants/materials, setting, methods: We considered the first oocyte donation cycle, without severe male factor, myomas, hydrosalpinx, Asherman syndrome, polyps or indication for preimplantational genetic diagnosis divided in two groups; patients with MA and no malformed uterus. MA group includes cycles of complete bicorporeal uterus (162), partial bicorporeal (30), bicorporeal septate (15), T shaped uterus (26), infantilis uterus (8), complete septate uterus (110), partial septate uterus (94) and hemi-uterus without rudimentary cavity (29).
Main results and the role of chance: We registered 58 342 patients from our oocyte donation program. Results are shown as mean and 95%CI and differences in pregnancy rates were expressed as relative risks (RR) with 95%

CI being reference patients with normal uterus. In patients with MA, the implantation rate was different according the categories being significantly lower in patients with unicornuate uterus (0.29 95%CI: 0.14-0.43. p=0.03). Biochemical pregnancy rate was significantly higher in patients with septate uterus (RR 1.51 (95%CI 1.02-2.22, p=0.03) and significantly lower in unicornuate uterus (RR 0.49 (95%CI 0.27-0.90). No differences were found in clinical pregnancy rate among groups, but ongoing pregnancy rate and live birth rate were lower in unicornuate uterus (RR 0.28 (95%CI 0.13-0.63, p= 0.002), (RR 0.32 (95%CI 0.14-0.73, p=0.007) respectively. Miscarriage rate was significantly higher in patients with septate uterus (RR 1.78 (95%CI 1.18-2.68, p=0.006)

Limitations, reasons for caution: As this was a retrospective cohort study, we were unable to study differences due to modifications in medical or laboratory protocols during this long period time. Different size of sample in some groups of MA makes impossible to translate conclusions to general population.

Wider implications of the findings: Our results indicate that there might be a defect in the embryo implantation rate in patients with MA depending on uterine factor. Different sample size among groups and some groups with scarce number of cases make less precise results. More studies controlling biases are needed to confirm our results.

Trial registration number: NCT04571671

P-330 Live birth after ART in patients with treated endometriosis versus those without endometriosis at laparoscopy

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Study question: Is endometriosis related to worse outcome of assisted reproductive techniques (ART)?

Summary answer: Cumulative incidence of live birth in patients with and without endometriosis at laparoscopy was similar, although deep endometriosis and adenomyosis were negative prognostic factors.

What is known already: Whether endometriosis has a negative impact on the outcome of ART is still a matter of debate. Most published data report on one fresh cycle only, usually without taking frozen embryos into account. Further, a large heterogeneity in study population has been acknowledged by several meta-analyses, as in the control groups endometriosis was not always excluded by laparoscopy, and in case of endometriosis the prior treatment history was variable or unclear.

Study design, size, duration: Retrospective longitudinal cohort study of 1462 patients (779 with laparoscopically treated endometriosis of any rASRM stage, and 683 without endometriosis at laparoscopy) undergoing ART treatment between July 2003 and December 2014. Primary outcome studied was time to ART live birth. Secondary outcomes include -amongst others- number of cycles needed per ART live birth, time to ART or spontaneous live birth, cycle cancellation rate, and pregnancy outcomes like miscarriage and ectopic pregnancy, and per cycle analyses.

Participants/materials, setting, methods: All patients with a history of laparoscopy prior to the start of their first ART were included for analysis. The ART was performed in a tertiary referral center of a large University Hospital. Primary outcome studied was the time from initiation of the first ART cycle to delivery of the first live born. Survival analysis was conducted using cumulative incidence functions and cause-specific hazards regression.

Main results and the role of chance: The study included 1462 patients who initiated 4537 ART cycles, of which 3672 (81%) fresh and 857 (19%) frozen cycles. The unadjusted hazard ratio (HR) of live birth was 1.01 (95% CI 0.88-1.16). After adjustment for potential confounders (age, maternal BMI, maternal smoking, secondary infertility, duration of infertility, anovulation, reduced ovarian reserve, tubal factor, male factor and therapy started before 2007) the HR was 0.99 (95% CI 0.86-1.14). Within the endometriosis population (n=779), covariate-adjusted analyses suggested that presence of adenomyosis (HR = 0.54; 95% CI 0.34-0.86) and a history of deep endometriosis (HR=0.74 – 95% CI 0.58-0.94) were associated with a lower cumulative incidence of live birth. In contrast, there was little support of an association with diagnosis of stage III/IV (HR=1.15; 95% CI 0.84-1.59) or a history of ovarian endometriosis (HR=0.97; 95% CI 0.72-1.30). Beside the effect of the different variables directly linked to endometriosis, maternal BMI (HR= 0.80; 95%CI 0.71-0.91) and smoking (HR = 0.69; 95%CI 0.52-0.92) also negatively affected live birth delivery rate per patient.

Limitations, reasons for caution: Practices and success rates of ART may have changed during the 11-year recruitment period. Restricting to women who underwent laparoscopy, while providing evidence of the presence or absence of endometriosis, may have induced selection bias. However, the advantage of this time period, was the high rate of pre-ART laparoscopy (+/-50%).

Wider implications of the findings: As deep endometriosis and adenomyosis represent negative prognostic factors within the endometriosis population, future studies should focus on optimalisation of ART in these subgroups.

Trial registration number: S57393

P-331 The #Enzian classification: A comprehensive non-invasive and surgical description system for endometriosis.

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Study question: *Is there a classification for a complete mapping of endometriosis, including anatomical location, size of the lesions, and degree of involvement that can be used with both, diagnostics and surgery?*

Summary answer: #Enzian classification improves in both, non-invasive diagnostic methods and surgical therapy for endometriosis as a universally usable classification system for all aspects of the disease.

What is known already: The most commonly used r-ASRM classification has certain limitations due to its incomplete description of DE, the complexity of the classification, and lack of reproducibility. In contrast, the Enzian classification, which has been implemented in the last decade, has proved to be the most suitable for the description of DE. However, since it does not include peritoneal and ovarian lesions and lacks a description of tubo-ovarian adhesions, it has not gained full acceptance. A combination of classification with different systems such as r-ASRM, EFI score and Enzian, may complicate classification of the disease due to overlaps and time-consuming documentation.

Study design, size, duration: The result is a consensus of a panel of renowned clinicians (working group), gynaecological surgeons and sonographers with extensive expertise in diagnosis and therapy of endometriosis. A first draft was written in 2019 by a joint effort of the first and last author and sent to all working group members. Taking all comments into account, a revised draft was then sent to all coauthors and repeated until a consensus was reached (9 revisions).

Participants/materials, setting, methods: Criteria used to invite the experts to participate in this consensus process included their having significant peer-reviewed publications in the field of diagnosis and management of endometriosis.

Main results and the role of chance: Our current proposal is the first of its kind to universally describe superficial and deep endometriosis, ovarian endometriosis, adenomyosis and adhesions by using a classification system that can be applied by gynaecologists, surgeons, sonographers and radiologists following the same principles. The correlation between preoperative and surgical staging, on the basis of the Enzian scheme, allows for consistent and clear classification of endometriosis, especially DE. Endometriosis can be mapped completely with one single classification system enabling the use of one common language.

Limitations, reasons for caution: This classification system is anatomically logical and should be easy to use. Further studies are ongoing and are needed to provide proof for the applicability, reproducibility and accuracy of the #Enzian classification for the description of endometriosis.

Wider implications of the findings: #Enzian classification now enabled better coverage of various endometriosis localizations. The possibility of using

this system preoperatively as well as postoperatively within the framework of diagnostics offers clinicians a significant improvement in the care of patients with such a complex disease.
Trial registration number: not applicable

P-332 Endometrium thickness is increased in gonadotropin stimulated IVF compared to unstimulated cycles, but this increase does not have a positive effect on pregnancy rate

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Study question: Is endometrial growth and endometrial thickness different in controlled ovarian stimulation (COS)-IVF compared to unstimulated cycles and does this have an effect on pregnancy rates?
Summary answer: Endometrial growth dynamic is different and endometrium is thicker in COS-IVF but this does not have a positive effect on pregnancy and live birth rates.
What is known already: Endometrial growth and endometrial thickness are a function of duration and concentration of estradiol (E2) stimulation. Endometrial thickness <8mm is related with lower pregnancy rates in IVF treatments. It is commonly assumed that an increase of endometrial thickness by increasing estrogen stimulation could have a positive effect on pregnancy rate. However, such a relationship has never been systematically analysed. Natural Cycle IVF (NC-IVF) is an ideal model to analyse the effect of high dose gonadotropin stimulation on several parameters such as thickness of endometrium and pregnancy rate.
Study design, size, duration: Retrospective single center, University based study including 235 COS-IVF and 616 NC-IVF cycles from 2015 to 2019. Polyfollicular COS-IVF cycles were only analysed until 09 2017 as embryo selection was introduced in Switzerland afterwards. Limiting the analysis to cycles without embryo selection enabled us to compare embryos derived from cIVF and NC-IVF. 1550 endometrial and 1068 E2 measurements were included in the analysis.
Participants/materials, setting, methods: Mean female age at the time when the cycles were performed was in NC-IVF 35.8±3.9y and in COS-IVF 34.9±4.2y (maximum 42y). Each woman performed on average 1.96±1.45 IVF cycles. Endometrial thickness and E2 serum concentrations were evaluated daily between day -4 and -2 (0=day of aspiration). Pregnancy and live birth rate were evaluated per transferred embryo. Statistically, student test and a repeated measure model and a logistic regression model both adjusted for age were used.
Main results and the role of chance: Endometrial thickness was different in COS-IVF and NC-IVF. At each time point endometrial thickness was found to be higher in COS-IVF compared to NC-IVF ($p<0.001$ on days -4,-3, and -2). On day -2, the day when ovulation was triggered, mean endometrial thickness was 9.75 ±2.05mm in COS-IVF and 8.12 ±1.66mm in NC-IVF.

Endometrial growth dynamic was also different in COS-IVF and NC-IVF. Endometrial thickness increased significantly faster in NC-IVF cycles (0.58mm/day [0.43,0.73]) than in cIVF cycles (0.22mm/day [-0.12, 0.55], Pval= 0.034). The increase of endometrial thickness per day was less pronounced if E2 concentrations were high (-0.19 [-0.34, 0.05]). Therefore it can be assumed that the observed differences in growth dynamics in both treatments are caused by differences in E2.

Increased endometrial thickness in COS-IVF was not associated with higher success rate. There was no significant effect of endometrium thickness on pregnancy (Pval=0.318) and Live birth rate (Pval=0.461).
Limitations, reasons for caution: Pregnancy and live birth rates might be affected by more than just endometrial thickness. The study was only based on the thickness of the endometrium but not on its ultrasound pattern.
Wider implications of the findings: Postponing the aspiration to allow endometrium to further proliferate has only a limited effect in COS-IVF. Increasing gonadotropin stimulation dosage just to increase endometrial thickness is not a feasible strategy to improve pregnancy rate. The need to apply high dosages of estrogen supplementation in thawing cycles need to be questioned.
Trial registration number: "not applicable"

P-333 Vaginal and endometrial microbiota: is there any correlation

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Study question: Is there any correlation between the total bacterial load and the lactobacilli quantities in the vaginal and endometrial microbiomes in reproductive-age women?
Summary answer: There was no correlation between the vaginal and endometrial total bacterial loads and only a weak positive correlation between the quantities of lactobacilli.
What is known already: The *Lactobacilli*-dominated microbiota is considered to be the most favorable type of microbiota in the uterine cavity. It is associated with increased reproductive success in women undergoing *in vitro* fertilization. Whereas the non-*Lactobacillus* dominated microbial communities are more frequent in women with poor pregnancy outcomes.

When analyzing endometrial microbiota, one of the challenges is sampling. Transvaginal sample intake involves the possibility of contaminating the samples with vaginal microbiota. Moreover, it is an invasive procedure leading to the development of infectious inflammatory diseases of the upper genital tract. Thus, researchers are currently searching for predictors of the state of endometrial microbiota.
Study design, size, duration: It is a cross-sectional study of the vaginal end endometrial micorbiomes from 64 reproductive-age women. Endometrial and vaginal samples were collected simultaneously on days 7–10 of the menstrual cycle. To avoid contamination by vaginal microbiota, Endobrush Standard for Endometrial Cytology (Laboratoire C.C.D.; France) was used for endometrial sampling.
Participants/materials, setting, methods: The study included women who came to the "Garmonia" Medical Center (Yekaterinburg, Russia) seeking infertility treatment. The average age of the patients was 32.2±5.0.

DNA from vaginal and endometrial samples was extracted using PREP-NA-PLUS kit (DNA-Technology, Russia). Vaginal and endometrial microbiota was analyzed using Femoflor real-time PCR kit and DTprime 4M1 thermocycler (DNA-Technology, Russia).
Main results and the role of chance: Total bacterial load (TBL) in vaginal discharge was 3.8–7.9 lg (median — 7.1, interquartile range — 6.6-7.4). TBL in the endometrial samples was 0–5.1 lg (median — 3.9, interquartile range — 3.6-4.2). There was no correlation between TBL values in vaginal discharge and endometrial samples (Spearman's rho — 0.247, p=0.049).

Lactobacilli quantities in vaginal discharge were 4.5–8.3 lg (median — 7.2, interquartile range — 6.4–7.6), in endometrial samples — 0–5.1 lg (median — 3.7, interquartile range — 3.1–4.2). There was a weak positive correlation between lactobacilli quantities in vaginal and endometrial samples (Spearman's rho — 0.362, p=0.003).

The proportion of lactobacilli in vaginal discharge was 1–100% (median — 100%, interquartile range — 95–100%), in the endometrial samples — 0–100% (median — 96%, interquartile range — 25–100%). There was no correlation between lactobacilli proportions in vaginal and endometrial samples (Spearman's rho — 0.225, p=0.074). Furthermore, there was no correlation between lactobacilli quantity in the vagina and their proportion in the endometrial microbiota (Spearman's rho — 0.294, p=0.018).

There was only a weak positive correlation between the quantities of lactobacilli in vaginal and endometrial samples. Vaginal TBL values and lactobacilli proportions did not correlate with lactobacilli quantities and proportions in the endometrial samples.
Limitations, reasons for caution: The study was conducted on a small sample. Moreover, it is notoriously difficult to interpret the analysis results for endometrial microbiota due to the high risk of contamination and its low microbial biomass.
Wider implications of the findxings: Apparently, there is no obvious link between the vaginal and endometrial microbiomes. It is possible that, apart from

vaginal microbiota, there are other predictors which could allow us to assume whether lactobacilli are present in the endometrial microbiota.
Trial registration number: not applicable

P-334 CT virtual Histerotomography: a new method for the evaluation of fallopian tube patency and pelvic organs in patients seeking pregnancy

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Study question: Can computerized virtual histerotomography (CT-HSG) be used for the evaluation of fallopian tube patency and pelvic organs in patients seeking pregnancy? Summary answer: CT-HSG seems to be an adequate test for the evaluation of fallopian tube patency, pelvic organs, and the uterine cavity.
What is known already: CT-HSG is a minimally invasive exam, which diagnoses variations in the female reproductive system, uses low radiation doses and is well tolerated by patients. It simultaneously evaluates the uterine wall, cavity and cervix, tubes, and adjacent pelvic structures. The exam enables virtual navigation, which consists of the endoluminal view of the cervical canal and uterine cavity and allows 3D reconstruction of images. The exam remains underused to assess infertility, but previous studies have shown potential and its use may be widespread.
Study design, size, duration: Retrospective cohort study, that included data from 317 women seeking pregnancy, between January/2019 and January/2021. The CT-HSG was indicated for infertility (90.3%) and RPL (0.9%) investigation, and for the evaluation of tubal stump in patients who were planning the tubal reversal surgery (8.8%). Patients filled out a questionnaire about their pain symptoms and data were collected from electronic records.
Participants/materials, setting, methods: The study analyzed patients' clinical characteristics and image findings regarding tubes, uterine cavity, and ovaries. For the exam, a catheter was positioned in the cervix, where the contrast medium (iopromide) was injected through an infusion pump at 0.30 ml/s, for a total of 20ml. The tomographic slices were obtained at the 50th second. The CT-HSG images were interpreted by the same gynecologist and radiologist. Data were analyzed using SPSS version 20.0.
Main results and the role of chance: Women and partners' mean age was 32.7 ± 5.6 and 34.6 ± 7.7 years, respectively, and women's mean BMI was 28.4 ± 6.4 Kg/m². The pain scale was applied in 103 patients, who reported 5.4±3.2 pain scale scores at the end of the exam. Among the infertile patients 67% were nulliparous. Regarding the exam findings, most of the uterus findings were normal (72.6%). The variations found were uterine malformations (including unicornuate uterus, uterus didelphys, bicornuate uterus, septate uterus, and arcuate uterus), synechia, fibroids, endometrial polyps, adenomyosis and retractions/lateralizations that may suggest endometriosis. The tubal findings on the right/left (%) were: 65/67.5 patent horn; 18.9/17.7 obstructed tubes; 4/41 dilatation/hydrosalpinx and 9.4/9.1 with previous history of tubal ligation or salpingectomy; 1.5% of the tubal evaluation were inconclusive. Eleven from 317 patients had to repeat the exam due to occurrences during the execution (for example, improper catheter positioning, cuff fall, stenosis of the internal cervical ostium, severe pain).The 3D analysis and virtual navigation assist in the findings assessment, in addition to being simpler for the gynecologists evaluation.
Limitations, reasons for caution: The sample size is small due to the exam being a new technique. Patient follow-up and correlation with laparoscopy and hysteroscopy, when indicated, are under studied.
Wider implications of the findings: The exam seems to be promising for assessing infertility, RPL and the tubal stump. Moreover, it may be a good option to hysterosalpingography as it seems to cause less pain and allows to evaluate the ovaries and the uterine contour, added to 3D reconstructions and to virtual uterine navigation.
Trial registration number: not applicable

P-335 Endometrial compaction 5-10% is associated with the best live birth rate in artificial frozen-thawed embryo transfer cycles

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Study question: What is the effect of endometrial compaction on live birth rate in frozen-thawed embryo transfer (FET) cycles?
Summary answer: In FET cycles with artificial endometrial preparation, the chance for live birth was significantly higher in cycles with endometrial compaction.
What is known already: Most studies conclude that thinner the endometrium poorer the pregnancy outcome. These studies mostly include measurements in the follicular phase. Since endometrial thickness indicates receptivity, one may expect the endometrial thickness measured on ET day to be more important to predict the outcome. However, few studies assessed endometrial thickness on ET day and unlike follicular phase studies conflicting results were obtained regarding pregnancy outcome. The change in endometrial thickness may be more valuable to predict the pregnancy outcome rather than a single measurement.
Study design, size, duration: Retrospective observational cohort study. 283 FET cycles in which all patients underwent artificial endometrial preparation were reviewed. Participants/materials, setting, methods: The inclusion criteria were artificial endometrial preparation, age between 20-38 years. The same protocol was applied to all patients for the endometrial preparation.The change of endometrial thickness between the end of estrogen phase and embryo transfer day was recorded. Any decrement is defined as endometrial compaction. The patients were grouped according to the changes of endometrial thicknesses as compaction and non-compaction.
Main results and the role of chance: Among 283 cycles, 89 had endometrial compaction and 194 did not have compaction. The clinical pregnancy, implantation and live birth rates were significantly higher in the compaction group when compared to non-compaction group (*P* values 0.007, 0.009, and 0.039, respectively). In order to evaluate the results according to the degree of compaction, we divided the patients into 5% compaction slices. The live birth rate was significantly higher in the 5-10% compaction group (P=0.016). A multivariable logistic regression analysis was performed to examine the independent effects of different variables on live birth chance.In FET cycles with artificial endometrial preparation, the chance for live birth was significantly higher in cycles with endometrial compaction (OR: 2.352, 95% confidence interval {CI} 1.297-4.264, *P*=0.005). A receiver operating characteristic (ROC) curve analysis was performed to evaluate whether there was a certain threshold of endometrial thickness at the end of estrogen phase for endometrial compaction to occur. The sensitivity and specificity of 9.25 mm at the end of estrogen phase calculated from the ROC curve were 76.4% and 58.8%, respectively (area under the curve: 0.701, 95% CI 0.640-0.763; *P*<0.001).
Limitations, reasons for caution: The main limitations of the study were its retrospective nature, relatively small sample size and utilization of different ultrasound techniques at different measurements (using transvaginal ultrasound at the end of the estrogen phase and transabdominal ultrasound on ET day).
Wider implications of the findings: Recently a cohort study they found that endometrial compaction results in better pregnancy outcomes, similar to our findings. But, this is the first study to suggest a threshold value (9.2) for endometrial thickness before the commencement of progesterone in regards to increase the chance of compaction.
Trial registration number: not applicable

P-336 The role of endoplasmic reticulum stress in endometriosis; preliminary results

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Study question: Can X-box binding protein 1 (XBP-1) be used in evaluation of endoplasmic reticulum (ER) stress in endometriosis patients?
Summary answer: High levels of XBP-1 among endometriosis patients indicate an association between ER stress and endometriosis.
What is known already: ER is responsible for protein folding, lipid synthesis, and calcium homeostasis. ER stress occurs due to the accumulation of unfolded or misfolded proteins in the ER. ER stress causes the activation of several signal transduction cascades, defined as the unfolded protein response (UPR). In the studies conducted with ectopic endometrial tissue and cells, it was reported that UPR plays a role in the pathogenesis of endometriosis. The XBP-1 is a transcription factor involved in UPR, where it regulates ER stress-mediated apoptosis. XBP-1 is also responsible for endometrial cell migration, which is also a part of the pathogenesis of endometriosis.
Study design, size, duration: This prospective case-controlled study was conducted at University of Health Sciences Turkey, Istanbul Kanuni Sultan Suleyman Training and Research Hospital Department of Obstetrics and Gynecology between March 2020 – August 2020. A total of 60 subjects were included in the study. All patients gave their written informed consent before their enrollment in the study.
Participants/materials, setting, methods: 30 endometriosis patients aged 18-45 years were included in the study group. Patients with a history of ovarian surgery, endocrine, autoimmune and metabolic disorders, and hormonal treatment during the previous three months were excluded. 30 healthy subjects without endometriosis were included in the control group. Endometriomas were measured with transvaginal ultrasonography and pain was evaluated with visual analogue scale (VAS). XBP-1 levels were determined from serum samples using Human XBP-1 ELISA Kit (Elabscience Co., USA).
Main results and the role of chance: The mean age of the control group was 28.33±2.49, and the study group was 27.76±2.45 (p=0.374). The mean endometrioma volume in the study group was calculated to be 9.9± 9.05. The mean XBP-1 level in the control group was 1008.31± 329.05, whereas this level in the study group was significantly higher (2710.65±1484.13, p<0.001). When the study group was divided according to VAS scores into two groups, the mean XBP-1 level, and endometrioma volumes were significantly higher in the group with VAS scores > 6 (p <0.001 and p=0.03 respectively). A receiver operating curve (ROC) analysis was conducted in the study group. The area under the curve AUC for XBP-1 levels was 91% (95%CI: 0.86–0.96, p <0.001) for the cut-off value of 1279.52 with a sensitivity 87.2%, specificity 86.7%, PPV: 90.4%, NPV: 82.5%, +LR: 6.5, -LR: 0.1. The AUC for VAS scores >6 was 96.2% (95%CI: 0.93–0.98, p<0.001) for the cut-off value 2227.71, with a sensitivity 90% and a specificity 91.1%, PPV: 87.1%, NPV: 96.1%, +LR: 10, -LR: 0.1.
Limitations, reasons for caution: A limitation of this study was the methodology of serum sample collection. Since there are no data available on the timing of sample collection with regard to the menstruation cycle of the subjects, samples were collected at the first consultation of the patients without considering the date of their cycle.
Wider implications of the findings: In this study, XBP-1 levels in the endometriosis group and also among patients with VAS scores of >6 were significantly higher. This association between XBP-1 and endometriosis and the positive correlation with pain indicates that XBP-1 can be a potential biomarker, especially in the presence of severe pain symptoms
Trial registration number: NCT04440397

P-337 Mid-Infrared spectroscopy as a real time diagnostic tool for chronic endometritis

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Study question: Can we develop a real-time diagnostic tool for chronic endometritis (CE) by using attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy to evaluate biopsies obtained during hysteroscopy?
Summary answer: A discrimination model based on the absorbance data was developed by machine learning techniques, differentiating between positive and negative CE histopathology with 97% accuracy.
What is known already: CE is diagnosed in approximately 15% of infertile women who undergo *in vitro* fertilization (IVF), in 42% of women with recurrent implantation failure (RIF), and in 57.8% of women with RPL. Diagnosis is done by endometrial biopsy, and the presence of plasma cells in the endometrial stroma is the generally accepted histological diagnostic criterion. However, the histological detection of CE is time-consuming and difficult. ATR-FTIR spectroscopy is a non-destructive method that can provide valuable information on biochemical changes that occur during pathological processes, such as inflammation and cancer.
Study design, size, duration: We performed a prospective study in which fresh biopsies of endometrium were obtained during standard hysteroscopies. Each biopsy was examined by the spectrophotometer and afterward by histopathological analysis in which multiple myeloma oncogene 1 (MUM-1) staining for plasma cells, a marker of CE, was performed. We planned to investigate 80 samples to develop a discrimination model, and another 40 samples for validation of the model. The study was planned to last two years.
Participants/materials, setting, methods: Women that underwent hysteroscopy as a part of infertility evaluation were recruited. The hysteroscopies and the biopsy evaluation were performed at the same center. A cut-off of 8 MUM-1 positive cells per 10 high power fields (HPF) was set. We compared the spectroscopy analysis of the positive CE group (≥8) and the negative CE group (<8). Machine learning technique was utilized to build discrimination models. Data analysis was performed using Matlab and Unscrambler software packages.
Main results and the role of chance: We present preliminary results for our study. Forty-two women were recruited from January 2020 until November 2020. Of the 42 measured spectra, three were discarded due to high measurement noise. Of the 39 biopsies, 33 had MUM-1<8 (CE negative group) and 6 had MUM-1≥8 (CE positive group). Measured spectra of tissue smears from CE negative and positive groups differed from each other in the spectral range of 850-990 [cm-1] (p<0.05). This wavenumber can be associated with the C-H in-plane bend in the alkene group (CnH2n). A discriminant model was developed between the groups using the Principal Component Analysis and Linear Discriminant Analysis techniques. The accuracy obtained by the model was 97%.

We divided the 39 hysteroscopies based on the CE signs into 2 groups: "Negative hysteroscopic-CE" and "Positive hysteroscopic-CE". Positive hysteroscopic signs were micropolyps, strawberry pattern, hyperemia, punctuation, or pale endometrium. Twenty-three samples were taken in the Negative group and 16 samples were taken in the Positive group. However, measured spectra of tissue smears from negative and positive hysteroscopy groups were not significantly different. The correlation coefficient between hysteroscopy groups and MUM-1 score was r=0.29, meaning that the characteristic signs of CE in hysteroscopy were not correlated to the histopathology.
Limitations, reasons for caution: First, these are preliminary results and we need to investigate more samples to validate our model. Second, diagnostic criteria for CE are diverse in the literature and we chose 8 MUM-1 positive cells in 10 HPF, a criterion which may not be accepted by all experts in the field.
Wider implications of the findings: ATR-FTIR spectroscopy is highly sensitive to molecular changes and has been utilized as a diagnostic tool in a variety of clinical studies. While histopathological results take about two weeks, ATR-FTIR spectroscopy might give us the possibility to diagnose CE in real-time, allowing an immediate initiation of the appropriate treatment.
Trial registration number: ClinicalTrials.gov Identifier: NCT04197167

P-338 Epigenetic alterations in H19-DMR regulatory region in endometrial tissues of women with endometriosis

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Study question: Is epigenetic modifications pattern in DMR region of *H19* gene different in endometrial tissues of women with endometriosis in compare to normal endometrium?
Summary answer: The methylation level in DMR region of *H19* gene was significantly lower in the endometriosis group.
What is known already: Endometriosis is characterized by the growth of endometrial-like tissue outside the uterus and has been considered as an epigenetic disease. The lncRNA H19 and insulin-like growth factor-2 (*IGF2*) genes form a reciprocally imprinted cluster (*IGF2/H19*). The expression of these two genes is regulated by imprinting control region (ICR). The ICR region is located between these genes and is a differentially methylated region (DMR). The *H19* and *IGF2* genes are involved in regulating cellular growth and differentiation and might be targeted by MeCP2 (a marker of DNA methylation) for subsequent epigenetic modifications through DMR regulatory region.
Study design, size, duration: In this case-control study, 12 endometrial samples (eutopic) and 12 endometriotic lesions (ectopic) of women with endometriosis and 12 endometrial control samples were analyzed. Control samples were obtained from women who had no evidence of endometriosis during diagnostic laparoscopy. Control and eutopic endometrial samples were obtained by pipelle. Ectopic samples were obtained during laparoscopy. All women signed the informed consent form and did not receive any hormonal treatments during the last three months.
Participants/materials, setting, methods: After endometrial tissues collection, gene expression levels of *IGF2* and *H19* were evaluated using real-time PCR . The occupancy of MeCP2 on two subregions within DMR region of *H19* gene was investigated using chromatin immunoprecipitation (ChIP) followed by real-time PCR. One-way ANOVA was used for data analysis. P value less than 0.05 was considered statistically significant.
Main results and the role of chance: Gene expression profile of *H19* and *IGF2* was decreased in eutopic and ectopic endometrial lesions of endometriosis group compared with control ones. The decrease in gene expression of H19 in ectopic samples was significant in compared to the control ones while it was nearly significant in compared to the eutopic samples (p=0.01, p=0.056, respectively). The ChIP analysis revealed that MeCP2 incorporation in region II (between −3945 and -3818 bp)within DMR region of *H19* gene was significantly decreased in eutopic samples compare to control group (p=0.02) while its decrease was nearly significant in ectopic samples (p=0.056). However, this DNA methylation profile was not significantly different between eutopic and ectopic endometrial samples in endometriosis in group. Incorporation of MeCP2 in region I (between −2230 and -2103 bp)within DMR region of *H19* gene was not significantly different between the eutopic, ectopic and control samples (p>0.05). (data was presented at 21th Royan International Congress).
Limitations, reasons for caution: The main limitations of this study is its small sample size. For getting more information, we need to monitor this DNA methylation profile in a large number of women with and without endometriosis. Also, more investigations are required to clarify the further epigenetic modifications in this region.
Wider implications of the findings: It seems that reduced expression of *IGF2* may be due to hypomethylation of H19-DMR region II while this hypomethylation has no effect on H19 expression in endometriosis. As previously was shown , hypomethylation of H19-DMR causes decrease of IGF2 expression and increase in H19 expression.
Trial registration number: not applicable

P-339 Fertility treatment and live birth are still possible following the unexpected diagnosis of endometrial carcinoma/complex hyperplasia - provided that there is careful multidisciplinary team involvement

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Study question: Is it safe for young women to delay hysterectomy for endometrial carcinoma or complex hyperplasia, have fertility treatment and carry a pregnancy to term?
Summary answer: Fertility treatment and livebirth are possible after a diagnosis of endometrial carcinoma or complex hyperplasia but close co-operation between fertility and gynae-oncology services is key.
What is known already: While predominantly a disease of postmenopausal women, 7% of cases of endometrial adenocarcinoma or complex hyperplasia occur in women under 40 years. The standard surgical treatment is hysterectomy, which is curative in the majority of cases. In younger women wishing to preserve fertility, conservative treatment may be considered. The fertility outcomes in this population are not well reported, possibly because fertility preservation is not always discussed or considered when faced with the devastating diagnosis of cancer or pre-cancer in the younger woman or because of concerns regarding the impact of pregnancy or ovarian stimulation on a predominantly oestrogen sensitive tumour.
Study design, size, duration: This case series retrospectively evaluated the outcomes of 6 women with endometrial adenocarcinoma or complex hyperplasia who attended Merrion Fertility Clinic, Dublin from 2013 to 2020 and who were managed conservatively. These women initially presented with a history of infertility for which they underwent routine ultrasonography, which then led to hysteroscopy and endometrial biopsy. The histopathology of all 6 women showed an incidental finding of endometrial adenocarcinoma or complex hyperplasia.
Participants/materials, setting, methods: Patient files and a fertility clinic online database were reviewed to identify those with a diagnosis of endometrial carcinoma or complex hyperplasia. Their treatment course and reproductive outcomes were followed up, as was there eventual definitive surgical treatment.
Main results and the role of chance: Six women attending our service over a 7 year period were found to have endometrial adenocarcinoma or hyperplasia. They ranged in age from 34 to 46(mean 39). All were nulliparous. Four of the women had adenocarcinoma and 2 had complex hyperplasia. One woman, aged 41, with grade II endometrial adencocarcinoma was deemed unsuitable for conservative management by the gynaecological oncology team. She underwent urgent total abdominal hysterectomy and is well. The remaining 5 women proceeded with conservative management with oral or local progesterone therapy for 6 to 12 months. This resulted in an inactive endometrium on follow-up endometrial biopsy. Once disease regression was achieved, assisted reproduction in the form of in-vitro fertilization (IVF) was advised to ensure minimal time to pregnancy. Two of the women conceived using own egg IVF and two with donor eggs. All were successful in achieving at least one live birth. One had twins and one had 2 singletons, from a fresh and a frozen embryo transfer. The 6th woman has embryos frozen but has not yet had embryo transfer. Two of the 6 women ultimately had a hysterectomy, while 4 continue to be followed up with 6 monthly endometrial biopsies and progesterone therapy.
Limitations, reasons for caution: This study is limited by the small sample size. However, this paper reports on a niche subset of the population and finding larger sample sizes would be difficult to obtain.
Wider implications of the findings: This case series illustrates the favourable outcome of pregnancy with IVF after either systemic or local progesterone therapy in early stage endometrial adenocarcinoma or complex hyperplasia. Early involvement of a fertility specialist may prove highly valuable in cases of fertility sparing treatment to increase each patient's potential for pregnancy.

Trial registration number: not applicable

P-340 Novel non-invasive diagnostic options for endometriosis - based on glycome analysis

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Study question: Could glycosylation changes on serum and/or urine glycoproteins be suitable biomarkers for the non-invasive diagnosis of endometriosis?
Summary answer: The glycosylation pattern on serum and urine glycoproteins differed significantly in endometriosis patients compared to controls, suggesting a novel role as biomarkers of the disease.
What is known already: There is little published on endometriosis and glycosylation, and most of the studies are conducted with tissue or peritoneal fluid samples, collected by invasive means. An Iraqi study draws attention to the importance of serum sialylation, which is dramatically changed in endometriosis patients after zoladex therapy, indicating that changes in serum sialylation may be a new biomarker of the disease. While glycosylation of urine in endometriosis has not been studied so far, in a study of endometrial cancer, the urinary level of two glycoproteins was significantly increased in the patients compared to the control group.
Study design, size, duration: This was a prospective study. In this basic research project, serum and urine samples were collected for glycome analysis in women with and without endometriosis, as diagnosed at laparoscopy. Glycated haemoglobin (HbA1c), fasting glucose levels as well as hormone levels were also collected from the patients to link our glycomic findings with metabolic and hormone profiles. The study was approved by the Research and Ethics Committee of the National Maternity Hospital, Dublin (EC19.2018).
Participants/materials, setting, methods: Samples from 24 cases of endometriosis (patients without previous anti-inflammatory or hormonal therapy, endometriosis was confirmed by laparoscopy) and 27 control patients (patients without endometriosis) were processed to analyse *N*-glycans (total serum), urine glycoproteins, and IgG. The pre-processed, PNGase F-digested serum and urine samples were labelled with fluorescent tag and then analysed by mass spectrometry, ultra-performance liquid chromatography (UPLC) in combination with exoglycosidase digestions and Glycostore (https://glycostore.org/).
Main results and the role of chance: Glycosylation on total serum and urine glycoproteins and IgG was investigated and differed in endometriosis compared to controls. The *N*-glycome from the total glycoproteins in serum and urine was also different. The proportion of the galactosylation and sialylation differed between urine and serum IgG and these alterations have an impact on the IgG function. Our preliminary data indicate, that there is an increase in alpha 2-3 sialylation, galactosylation, and fucosylation on urine glycans from endometriosis patients compared to the control pool. Urine is a good source of biomarkers as it can be collected non-invasively. Our group is the first to have developed a protocol for the recovery of *N*-glycans in urine and to have identified the total *N*-glycome in urine. The urine *N*-glycome contains mostly complex *N*-glycans and also some oligomannosylated and hybrid glycans. Our results may lead to non-invasive biomarkers for the diagnosis of endometriosis and the monitoring of the disease.
Limitations, reasons for caution: The number of participants involved in this basic research is low but this is a pilot study. A larger, validation study, is warranted in the future. Furthermore, the follow-up of treated patients also would be an interesting field of research.
Wider implications of the findings: Glycomics may be a potent source of biomarkers of endometriosis, with a number of glyco-biomarkers already approved by the FDA. Endometriosis-associated glycomic profiles from serum and/or urine glycoproteins may represent viable targets for development of innovative non-invasive or minimally invasive diagnostics in this debilitating disease.
Trial registration number: not applicable

P-341 Epigenetic role of the H2BK5ac histone modification on the expression of ALX1 and PDHX genes in the endometriotic tissues and normal endometrium

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Study question: Evolution of the *ALX1* and *PDHX* genes expression and incorporation of H2BK5ac mark on the promoter of these genes in endometriotic tissues *versus* normal endometrium
Summary answer: Lower incorporation of H2BK5ac mark in *ALX1* and *PDHX* promoters can because to downregulation of these genes in endometriotic tissues compared to normal endometrium.
What is known already: Endometriosis is considered as multifactorial disease affected by genetic, hormonal, and environmental factors. Recent evidences suggest the role of epigenetic mechanisms in this disease. Aristaless-like homeobox1 (*ALX1*) and Pyruvate Dehydrogenase Protein X (*PDHX*) genes are considered in this study. Studies show that upregulation of the *ALX1* gene cause cell proliferation, migration, and invasion in cancer cells. *PDHX* is involved in cellular metabolism and acts as a tumor suppressor gene while maintaining normal homeostasis. It is Hypothesized that H2BK5ac which is known as a dynamic marker in promoter regions of active genes, may be involved in regulation of these gene expression.
Study design, size, duration: Ten eutopic and ectopic endometrium tissue, as well as ten normal endometrium, were collected. Ectopic biopsies were obtained using diagnostic laparoscopy, while the endometrial control samples and eutopic samples were collected via pipelle.
Participants/materials, setting, methods: RNA extraction and cDNA synthesis were done then the expression of *ALX1* and *PDHX* genes evaluated by quantitative real-time PCR. Promoter regions of mentioned genes investigated for the incorporation of the epigenetic mark of H2BK5ac using Chromatin immunoprecipitation (ChIP) followed by real-time PCR. Data analysis performed using One-way ANOVA analysis (SPSS software) considered the significant level of $P < 0.05$.
Main results and the role of chance: Results showed that the expression of *ALX1* was significantly decreased in eutopic endometrial samples compared to normal endometrium ($p = 0.007$). Also, there was a significant reduction in PDHX mRNA level in the eutopic and ectopic samples *vs*. normal endometrium ($p = .017$ and $p = .021$, respectively). The chromatin immunoprecipitation real-time PCR (ChIP PCR) analyses showed significantly lower incorporation of H2BK5ac epigenetic mark in *ALX1* promoter in eutopic endometrial samples compared to normal endometrium ($p = 0.007$). Also, reduced incorporation of H2BK5ac at *the PDHX* promoter region was observed in both eutopic and ectopic endometrial samples compared to normal endometrium ($p = 0.004$ and $p = 0.003$, respectively).
Limitations, reasons for caution: The main limitation of our study is the low number of samples.
Wider implications of the findings: Our results suggest that the marked lower levels of H2BK5ac in regulatory regions of *ALX1* and *PDHX* might lead to deregulation of these genes in tissue endometriotic samples.

Trial registration number: 'not applicable' for non-clinical trials

P-342 Are blastulation and clinical pregnancy rates in women with endometriomas different than those without?

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Study question: Does the presence of endometrioma during ovarian stimulation affect blastulation and clinical pregnancy rates (CPR)?
Summary answer: Blastulation rates were similar in women with endometrioma compared to women without. Likewise, CPR were comparable.
What is known already: Although relationship of endometriosis and subfertility is well-established, its mechanism is still under investigation. Decreased oocyte quality, resulting from anatomical and/or inflammatory factors is one of the prominent culprits. Most studies regarding endometriosis and oocyte quality are highly heterogeneous and effect of endometriosis on oocyte quality is yet to be determined. Blastulation is thought as a surrogate marker for oocyte quality. Thus, it may be possible that detrimental effect of the presence of endometrioma during ovarian stimulation can be indirectly assessed by blastulation.
Study design, size, duration: Records of all women who underwent assisted reproductive technology treatment at Koc University Hospital Assisted Reproduction Unit between 2016 and October 2020 were screened for this retrospective study. All women who had endometrioma(s) during ovarian stimulation were included in the study group (EG) (n=71). They were matched with women diagnosed with tubal factor or unexplained infertility who underwent oocyte pickup within the same period to form the control group (CG) (n=104).
Participants/materials, setting, methods: All women underwent antagonist or long protocol. All embryos were cultured until blastocyst stage regardless of the number of oocytes or embryos available. Size/location of endometriomas, number of oocytes retrieved, number of available blastocysts, positive pregnancy test per cycle and clinical pregnancy rate per cycle were recorded. Blastulation rate was calculated as number of available blasts divided by the number of metaphase-II oocytes. Embryos were transferred in a fresh or artificially prepared frozen-thawed cycle.
Main results and the role of chance: There were 71 women in EG and 104 women in CG, which included 30 women with tubal and 74 with unexplained infertility. Median endometrioma size was 26 mm(22-33). Twenty-three patients in EG had history of endometrioma excision (31.3%). Median age [35.0 years (31.0-39.0) vs 34 (32.0-36.0), p=0.26] and serum AMH levels [1.8 (1.1 - 4.2) vs 2.3 (1.3 - 3.7) ng/dL, p=0.91] were similar in EG and CG, respectively. Body mass index in kg/m2 [21.8 (20.2-24.6) vs 24 (21.5-27.9), p<0.01] and infertility duration in years [2 (1-2.6) vs 3 (2-5), p<0.01] were significantly lower in EG. Number of retrieved oocytes [8 (5-12) vs 12 (7-15.8), p<0.01)] and metaphase-II oocytes [6 (4-10) vs 8.5 (6-12), p<0.01] were lower in EG group compared to CG group. However, blastulation rate per MII oocyte were similar between the EG and CG [(0.25 (0.20-0.41) vs 0.30 (0.14-0.50), respectively, p=0.58]. Adjusted analysis for age and number of MII oocytes revealed similar finding.

Positive pregnancy test per cycle was similar at 53.5% vs 61.5% in EG and CG, respectively (p=0.3). CPR were similar between the EG and CG (45% vs 58%, respectively, p=0.10).
Limitations, reasons for caution: Retrospective design, lack of live birth information are the main limitations of our study.
Wider implications of the findings: Presence of endometrioma during ovarian stimulation does not seem to adversely affect blastulation rates. While this is reassuring regarding oocyte quality, further research is required to assess its effect on live birth.
Trial registration number: Not applicable

P-343 Identification of circulating leukocyte microRNA-125b and microRNA-142-3p expression profiles in women with endometriosis before surgery and at 1 month after surgery

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Study question: To investigate the expression profiles of microRNA-125b and micrpRNA-142-3p in women with endometriosis, compared with controls before surgery and at 1 month after surgery.
Summary answer: The over-expression of miRNA-125b and miRNA-142-3p may be involved in the aetiopathogenesis of endometriosis which is related to systemic chronic inflammation.
What is known already: Currently, there is no reliable non-invasive diagnostic biomarker for endometriosis. MicroRNAs (miRs) are small, non-coding RNAs that are involved in the post-transcriptional regulation of gene expression and promising biomarker candidates for noninvasive diagnosis of various diseases. Despite the small number of studies found that miRNA-125b and miRNA-142-3p have been associated with endometriosis, further evidence is therefore required from studies that examine these two miRNAs in diagnosis and even follow-up of women with endometriosis. Owing to endometriosis is a systemic chronic inflammatory disease, investigating these miRs in blood leukocytes of patients with endometriosis may illustrates the molecular mechanism of endometriosis.
Study design, size, duration: This is a prospective longitudinal study performed between 2018 November and 2021 February. The sample size of 42 individuals of two groups were calculated considering the power analysis (α=0.05) with Mann-Whitney U test (effect size,d=0.8) and were calculated as 80%. Women with endometriosis (n= 21) and surgically confirmed endometriosis-free women (n=21) were included in the study. Laparoscopy and/or laparotomy was performed to determine the presence or absence of endometriosis.
Participants/materials, setting, methods: Women aged 18-50 years were recruited from two tertiary hospital settings. Severity of endometriosis was assessed by the rASRM classification. Using real-time quantitative PCR, miRNA-125b and miRNA-142-3p in leukocyte were analyzed in women with endometriosis before surgery and at 1 month after surgery, compared to controls without endometriosis. The results were calculated as relative quantification values. The presumed targets of these two miRNAs were identified via 3 different target prediction algorithms:TargetScan, miRDB and DIANA-TarBase.
Main results and the role of chance: There were no demographic discrepancies between groups. The relative expression of miRNA-125b and miRNA-142-3p were significantly higher in women with endometriosis than in control subjects before surgery (p=0.0001;p=0001) and at 1 month after surgery, respectively (p=0.0001;p=0001). Despite the relative expression of miRNA-125b and miRNA-142-3p were decreased 1.75- and 2.4-fold, respectively at 1 month after surgery, we observed no significant differences in the relative expression of miRNA-125b and miRNA-142-3p between before surgery and at 1 month after surgery, respectively (p=0.110; p=0.910). Bioinformatic analyses of three databases showed that miRNA-142-3p expression levels were found to be closely associated with fifty-seven genes. Among these target genes, *CFL2*, *RGL2*, *WASL*, *CRK*, *BNC2*, *CLOCK*, *TGFBR1*, *CIITA* and *ZNF217* were found to be associated with endometriosis. Whereas, no target gene were observed to be associated with miRNA-125b expression in common with these three databases. The ROC curves showed that the expression of miRNA-125b and microRNA-142-3p had an area under the ROC curve (AUC) of 0.77 (sensitivity 61.9%, specificity 88.1% (0.0001;95%CI 0.65-0.90)) and AUC of 0.71 (sensitivity 52.4%, specificity 73.8% (p < 0.007;95%CI 0.58-0.84)) before surgery, respectively. Correlational analysis showed a significant positive correlation between miRNA-142-3p and Hemoglobin A1c (Spearman's correlation, r=0.507;p=0.019) in the endometriosis group.
Limitations, reasons for caution: Further studies are needed to examine the expression of these miRs with a long-term follow up in order to increase their usefulness as a predictor in the clinical practice. It is required to identify the expressions levels of predicted target genes which are associated with endometriosis and regulated by miRNA-142-3p.
Wider implications of the findings: Findings suggest that the over-expression of miRNA-125b and miRNA-142-3p may be potential mechanisms involved in the etiopathogenesis of endometriosis which is a systemic chronic

inflammatory disease. We observed that miRNA-125b may be a more reliable biomarker than miRNA-142-3 for noninvasive diagnosis of endometriosis and even follow-up of women with endometriosis.
Trial registration number: 118S298

P-344 Clarifying tubo-ovarian abscess management: a risk score for predicting antibiotic failure

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Study question: Can antibiotic treatment failure of tubo-ovarian abscesses (TOA) be predicted based on clinical features at the time of diagnosis?
Summary answer: We propose a risk score including patient temperature, c-reactive protein and TOA size that could predict which patients are likely to fail parental antibiotic treatment.
What is known already: Current guidance is that the first line management of non-ruptured TOA is with parental antibiotics. However, it is reported that treatment failure rate is 20-30%. Alternative treatment modalities include radiological drainage or laparoscopic/open surgery. In patients who require intervention, outcomes, such as morbidity, length of hospital stay and fertility, are improved when this is performed early rather than later in their hospital admission. However, our current guidance is scant with regards to the decision making for interventional TOA management.
Study design, size, duration: This is a multicentre retrospective cohort study over 81 months (01/01/13- 30/09/19) identifying 214 consecutive patients admitted to hospitals in North-West London with diagnosed TOA. Participants/materials, setting, methods: Demographics, medical history, presenting symptoms, laboratory results, radiological findings, treatments administered, hospital length of stay and follow up data was collected. The patients were chronologically split with the first 150 being used for the development of our risk score. Univariate and bivariate analyses were employed to ascertain statistically significant variables in the failure of parental antibiotic. The remaining 64 patients were used for risk score validation.
Main results and the role of chance: Statistically significant variables were: temperature at admission (median= 37.1 °C vs 38.2 °C, p=0.0001), C-reactive protein (CRP) at admission (151mg/L vs 243mg/L, p=0.0001) and size of TOA (6.0cm vs 8.0cm, p=0.0001). Those requiring intervention, stayed in hospital twice as long as those who did not (4 days vs 8 days, p<0.001). A scoring system was formulated using the statistically significant variables. A score of ≥4 was associated with requiring radiological/surgical intervention (p<0.001), with sensitivity 69% and specificity 88% (AUC 0.859) when tested on the validation cohort.
Limitations, reasons for caution: Being a retrospective study, which puts the data at risk of information and selection bias. Although there are merits to a multi-centre study, variation in patient management will invariably cause data heterogeneity.
Wider implications of the findings: TOA patients may have their hospital management tailored early according to the postulated tool, alleviating uncertainty in their treatment as well as possibly reducing morbidity and length of hospital stay.
Trial registration number: not applicable

P-345 Reproductive outcome after treatment of chronic endometritis in patients with recurrent implantation failure

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Study question: Does the treatment of chronic endometritis (CE) improve reproductive outcome in patients with recurrent implantation failure (RIF)?
Summary answer: Treatment and resolution of CE seem to improve pregnancy rates in patients with implantation failure and CE.
What is known already: Over the last 10 years, the interest in the study of CE has increased given its important association with reproductive failure. The main cause of CE is an infection of the endometrial cavity caused by common microorganisms. Therefore, the recommended treatment is antibiotic therapy. Numerous studies demonstrate an improvement in reproductive outcome in patients with treated and resolved CE.

The objectives of this study are to evaluate the resolution rate of CE after antibiotic treatment in patients with implantation failure diagnosed with CE and to analyse their reproductive outcome after treatment.
Study design, size, duration: In this prospective case series, all patients with RIF who underwent diagnostic hysteroscopy, IHC analysis with CD138 of an endometrial biopsy and microbiological analysis of an endometrial sample between October 2018 and February 2019 were included.
Participants/materials, setting, methods: Hysteroscopic findings suggestive of CE were collected and endometrial biopsies were taken for pathological study with CD138 and microbiological assessment. Likewise, treated endometrial samples and the results of hysteroscopy or control biopsies after treatment were collected. The data of embryo transfers post-treatment were also included in our study.
Main results and the role of chance: 30 patients with implantation failure were included. 15 patients (50%) were diagnosed with CE using any of the aforementioned diagnostic methods. All diagnosed patients were treated with antibiotic therapy: positive microbiological samples (9) were treated according to our antibiogram whereas those samples who were negative but were confirmed to have CE by hysteroscopy or pathological assessment (6) were treated with doxycycline. In all cases, CE resolved after treatment, except only one patient who required a second course of antibiotics to acquire a negative result. Ten patients underwent an embryo transfer after resolution of CE, resulting in 60% of ongoing pregnancies.
Limitations, reasons for caution: Although our results are encouraging and in accordance with other studies, we are aware that this is an observational non-randomised study with a limited number of patients.
Wider implications of the findings: It seems that the treatment of CE, following its diagnosis using the aforementioned methods, can improve pregnancy rates in patients with RIF and CE. Therefore, it is likely recommendable to study CE using these three tests.
Trial registration number: Not Applicable

P-346 Luteal endometrial immunity and implantation rates.

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Study question: Does the percentage of CD56 + NK cells, CD 156 + D16 +, and the presence of CD 138 correlate with pregnancy rates?
Summary answer: Presence of CD 138, as a marker of chronic endometritis, correlates with implantation failure in patients submitted to endometrial immunological analysis despite being treated.
What is known already: Embryo-uterine cross-talk during implantation is a complex process, which involves the synchronization between hormonal aspects, changes in the endometrium, morphological and molecular embryonic quality,

and immunological aspects of the endometrium.The presence of CD 138 has been used as a marker of chronic endometritis, which is associated with lower rates of endometrial implantation since the presence of plasmocytes is believed to lead to an increase in interleukins harmful to embryonic implantation.
Study design, size, duration: Retrospective study, with 85 patients submitted to luteal endometrial biopsy, with the same endometrial preparation performed for embryo transfer, from Jun / 2019 to Oct / 20.
Participants/materials, setting, methods: Patients submitted to endometrial preparation, with estradiol, and endometrial biopsy performed on the fifth day of progesterone use. Immunohistochemistry was performed for CD 138, CD 56+, CD16 +. Patients with CD 138+ were treated with antibiotic therapy. Endometrial preparation performed after adequate treatment, and blastocyst transfer. Exclusion criteria: non-treated hydrosalpinx, endometrial adherences, submucosal fibroids, and endometrial thickness less than 5mm. A chi-square test was used to compare the implantation rate group and the p-value of 5% is considered significant.

Main results and the role of chance: The average age of patients with positive beta hCG was 35.63 years ± 8.00, and among patients with negative beta hCG was 39.31 ± 2.206, with p = 0.093.

The pregnancy rate was 55%, 1 blastocyst embryo was transferred, with 47 patients with positive beta hCG, of these, 10 were biochemical pregnancies, and 5 abortions, with 32 evolutionary pregnancies.

There was no difference between the percentage of NK CD 56+ cells among patients with positive beta hCG, with an average of 9.56% ± 11.65%, and 8.26% ± 7.32% among patients who did not become pregnant. (p = 0.694).

There was also no difference between patients regarding the number of NK CD 16+ cells, with an average of 7% ± 8.01%, in patients with positive beta hCG, and in patients with negative beta hCG, 4% ± 3,48%, ep = 0.174.

16 samples presented CD 138+, were treated with antibiotic therapy, but even so, there was a correlation with negative beta hCG and the presence of CD 138+ with p = 0.049.
Limitations, reasons for caution: A retrospective study with a low sample size.
Wider implications of the findings: More prospective studies are necessary to elucidate the effect of endometrial immunity and the influence of CD 138 on embryonic implantation.
Trial registration number: Not Applicable - Retrospective cohort

P-347 A comparative RCT of Intrauterine-GCSF versus Subcutaneous-GCSF in Thin Endometrium in IVF-ICSI Cycles

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Study question: Does GCSF by intrauterine route leads to better result in the treatment of thin endometrium as compared to GCSF by the subcutaneous route, in IVF-ICSI Cycles?
Summary answer: Yes, GCSF by intrauterine route leads to better result in the treatment of thin endometrium as compared to subcutaneous-GCSF, in ART Cycles?
What is known already: GCSF, is a member of the colony stimulating factor family of cytokines and growth factors. GCSF receptors are expressed in high concentration on dominant follicle, particularly at preovulatory stage.The endometrium also shows an increased expression of these receptors. GCSF concentration rises in the follicular fluid at the same time. Serum levels of GCSF are found to be in direct correlation with levels of GCSF in follicular fluid. Serum levels increase progressively from the day the embryo-transfer to the day of implantation. GCSF has been found to be beneficial in patients with thin endometrium and recurrent implantation failure.
Study design, size, duration: This was a RCT conducted between 2018-2019. 30 patients with thin endometrium were enrolled in each group. In either group, GCSF was given if endometrium was less than 7mm on day 14, maximum of two doses were administered. Patients undergoing frozen embryo transfer were recruited in the study, after meeting the inclusion and exclusion criteria. Primary outcome measured was increase in endometrium thickness and the secondary outcome was the clinical pregnancy rate and abortion-rate.
Participants/materials, setting, methods: 60 patients with thin endometrium were randomly divided into two groups. Group A: Inj. GCSF (300 mcg/1 ml) subcutaneously on Day 14 onwards alternate days for two doses. Group B: Inj. GCSF (300 mcg/1 ml) instilled slowly into the uterine cavity using an intrauterine insemination (IUI) catheter under USG guidance. Endometrial thickness was assessed after 48 h. If endometrial thickness was found to be <7 mm, a second infusion of GCSF was carried out.
Main results and the role of chance: In the subcutaneous group (group-A) the mean endometrial thickness before GCSF injection was 5.8 ± 0.6 mm and, after injection it increased to 6.9 ± 0.4 mm. Similarly, in the intrauterine group (group-B) the mean endometrial thickness before GCSF was 5.9 ± 0.7 which increased to a mean of 7.9 ± 0.5 after GCSF instillation. The difference between endometrial thickness before and after intrauterine infusion of GCSF was more than that in the subcutaneous group. In group-A, 08 patients conceived out of 30 patients (clinical pregnancy rate 26.6%) and in group B 11 conceived out of 30 patients in whom GCSF was instilled intrauterine (pregnancy rate 36.6%). Thus, there was a difference in the clinical pregnancy rate in the two groups, the intrauterine group yielding a higher clinical pregnancy rate, but it was not statistically significant. Because of the thin endometrium, we found an abortion rate of 25% (2/8) in the subcutaneous-GCSF group, and an abortion rate of 18% (2/11) in the intrauterine GCSF group.
Limitations, reasons for caution: There are few potential limitations because of the small sample size. Confounders such as obesity, smoking and alcohol intake, presence of adenomyosis and endometriosis, were not taken into consideration. Though prevalence of obesity is usually low in Indian women. Habits of smoking and alcohol are exceedingly uncommon in Indian women.
Wider implications of the findings: Use of GCSF plays an important role in management of patients of thin endometrium undergoing embryo transfer. It is an easily available and economical preparation in developing countries and the intrauterine instillation of GCSF can be easily practiced in an ART unit with good results in resistant thin endometrium patients.
Trial registration number: not applicable

P-348 Laparoscopic radiofrequency thermal ablation for diffuse adenomyosis: symptomatology after a long-term follow-up

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Study question: Is conservative laparoscopic treatment with RFA (radiofrequency thermal ablation) related to a good outcome on a long-term follow-up?
Summary answer: RFA for diffuse adenomyosis was related to a good outcome on a long-term follow-up in terms of pain and ultrasonographic reduction.
What is known already: Uterine adenomyosis may cause symptoms refractory to medical treatment. New, uterine-sparing treatments have been introduced for patients who desire avoiding hysterectomy. Among surgical techniques used for this purpose, radiofrequency thermal ablation (RFA) has been introduced, first for the treatment of uterine fibroids and then for focal adenomyosis. Diffuse adenomyosis is characterized by an extensive involvement of uterus, as on ultrasound less than 25% of the lesion is surrounded by healthy myometrium. It often leads to enhanced uterine volume, which presents soft consistence and globular aspect. Conservative treatment of diffuse adenomyosis is a real challenge.
Study design, size, duration: All consecutive patients who underwent RFA for diffuse adenomyosis in our institution between July 2011 and August 2017. Patients with focal adenomyosis were not included in the study. The treatment was reserved to selected patients who wanted to conserve the uterus and presented symptoms such as pain or abnormal uterine bleeding refractory to medical treatment. In all cases the treatment was performed by laparoscopy, which allowed for complete removal of extrauterine endometriosis, if associated.
Participants/materials, setting, methods: Nineteen patients (aged 33-49, mean 40) underwent radiofrequency thermal ablation for diffuse adenomyosis, and all of them completed the follow-up. Setting: referral center for endometriosis (Department of Obstetrics and Gynecology, Gynecologic Oncology and

Minimally-Invasive Pelvic Surgery, International School of Surgical Anatomy, Negrar). Follow-up consisted on ambulatory clinical evaluation with pelvic ultrasound and assessment of pain using the visual analog scale (VAS) ranging from 0 to 10 points for all pain components. Main results and the role of chance: Endometriosis was associated in12 cases, (63%) and in all cases was removed completely during surgery. The mean follow-up was 64 months (range 29-105). Abnormal uterine bleeding was present in 11 (60%) patients before the treatment and only in four of them (21%) during the follow-up. Preoperative and postoperative mean VAS score for dysmenorrea, dyspareunia, dyschezia and chronic pelvic pain was 6.95 vs 3.7, 4.1 vs 1.4, 3.7 vs 0.9 and 3.9 vs 1.5 respectively ($p<0.05$ for all pain components). The reduction of adenomyosis on ultrasound was observed in 75% of cases. After surgery, two of four patients who desired pregnancy conceived, one of them delivered at term by caesarian section and one had an extrauterine pregnancy. Hysterectomy was performed in two cases during follow-up, at 35 and at 84 months after RFA.

Limitations, reasons for caution: The present study reports outcome in a limited population as the treatment was reserved to selected cases. The results, particularly regarding fertility and pregnancy outcome should be taken with caution because of small numbers. In our opinion for the moment the treatment should be performed in selected cases.

Wider implications of the findings: The present treatment could be performed to avoid hysterectomy, as it was necessary only in two cases in our study. No cases of hysterectomy were reported within the first two years from surgery, so we can consider that RFA allows at least a temporary benefit on symptoms.

Trial registration number: not applicable

P-349 Does concomitant autoimmunity affect IVF/ICSI outcomes in women with endometriosis? A retrospective observational study

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Study question: To investigate differences in In Vitro Fertilization (IVF)/Intracytoplasmic Sperm Injection (ICSI) outcomes between endometriosis women who do or don't have a concomitant autoimmune disease.

Summary answer: Despite a higher oocyte yield, a trend for reduction in clinical pregnancy rates was observed in the autoimmunity group compared to women without concomitant autoimmunity.

What is known already: Endometriosis is an inflammatory chronic gynaecological disorder with a known detrimental impact on fertility. Endometriosis pathogenesis is still unclear. It has been postulated a role of both innate and adaptive immune system. The coexistence of endometriosis and autoimmunity is a well-documented occurrence Some recent findings have revealed an increased risk to have concomitant autoimmune disease in women with endometriosis, but no study has so far investigated whether this association could affect IVF/ICSI outcomes. Indeed, autoimmune phenomena, including proinflammatory cytokines and auto-antibody production, may result in diminished quality of oocytes/embryos with lower pregnancy rates among these patients.

Study design, size, duration: This was a retrospective observational study carried out at the Fertility Unit of IRCSS San Raffaele Hospital (Milan). We reviewed medical patients' notes of women with a confirmed diagnosis of endometriosis who referred to our Fertility Unit from October 2018 to January 2021.

Participants/materials, setting, methods: Out of 1441 patients undergoing IVF/ICSI, 98 women had surgical/histopathological diagnosis of endometriosis. 25 of them had a clinical and/or serological diagnosis of autoimmunity. Autoimmunity was assessed by clinical data (blood tests for auto-antibodies or rheumatological records) obtained from the electronic patient files stored in the database of our Fertility Centre. Clinical pregnancy was defined as the presence of at least one intrauterine gestational sac with a viable embryo at week 6 after transfer.

Main results and the role of chance: 25/98 (25.5%) endometriosis women with a concomitant autoimmune disease (cases) were compared with 73/98 (74.5%) endometriosis patients without autoimmunity (controls). The mean age was 37.36±3.63 and 36.93±3.79 (p=.623) in cases and controls respectively. The mean number of oocytes retrieved was higher in cases (5.78±4.07) than in controls (3.82±2.69;p=.041); similarly, cases showed an higher number of embryos (2.13±1.93 vs. 1.19±1.37;p=.041) and blastocysts (1.89±2.02 vs. 0.85±1.61;p=.041) obtained. A total of 47 fresh embryo transfer (ET) were performed. Considering all the endometriosis patients, the clinical pregnancy rate (CPR) per cycle was 34.0% (16/47); when stratifying for the presence of autoimmunity the CPR was 23.1% (3/13) in cases, and 38.2% (13/34) in controls (p=.494).

Limitations, reasons for caution: This is a retrospective study based on data extraction from electronic records of our Fertility Centre. The sample size is limited and some information about past medical history could be missed. Results should be interpreted with caution until validated by future research providing more standardized data collection.

Wider implications of the findings: Despite significantly higher numbers of oocytes retrieved and embryos/blastocysts formed, the presence of concomitant autoimmune disease in patients with endometriosis may impair pregnancy rates. Whether this finding is confirmed and whether it could be due to a defect in embryo/blastocysts quality or in endometrial receptivity deserves further studies.

Trial registration number: not applicable

P-350 Utilizing indocyanine green (ICG)-enhanced fluorescence to localize the ureter during robotic surgery for DIE and a concomitant crossed renal ectopia

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Study question: Feasibility of (ICG)-enhanced fluorescence in visualizing the atypical course of the ureter during surgery for (DIE) of pelvic sidewall with a concomitant crossed renal ectopia.

Summary answer: Near-infrared fluorescence after transurethral injection of ICG enables localization of the ureter during surgery, thus facilitating complete excision of the lesions while enhancing patient's safety.

What is known already: Existing case series refer to the transurethral injection of ICG and visualization under near-infrared (NIR) light during robotic surgery for real-time delineation of the ureter, which helps to prevent iatrogenic ureteral injury during complex surgery. The ICG reversibly stains the inside lining of the ureter by binding to proteins on urothelial layer. The consequent green fluorescence allows its identification throughout the entire case.

The presented case of a DIE of pelvic side wall along with an ipsilateral concomitant crossed renal ectopia (residual function 27%) resembles an utmost challenge for surgery. To our knowledge no similar case has been reported in literature.

Study design, size, duration: Demonstration of the Robotic technique by means of a step-by-step tutorial

Participants/materials, setting, methods: 29-year-old patient referred after preceding laparoscopic surgery for DIE of left pelvic sidewall and abortion of surgery due to lack of accessibility/ severeness of the case. We performed renal scintigraphy, pelvic MRI and urological consultation.

Surgery was performed using an XI-da-Vinci robotic system. After cystoscopic placement of mono-Js we injected 4 ml. of ICG-solution (2,5 mg/ml). Visualization of the pelvic kidney was achieved 4 minutes after injection and of the complete ureter after 7-8 Minutes.

Main results and the role of chance: The robotic surgery could be completed safely and achieve a complete resection of the DIE of the pelvic sidewall including adhesiolysis of a broadly adherent bowel, opening of the rectovaginal space, ureterolysis of the distal portion of the ureter, partial excision of the left sacrouterine ligament and deperitonealization of the pelvic sidewall.

Postoperative controle revealed normal renal function and an adequate postoperative course.

Limitations, reasons for caution: ICG cannot be used in patients with iodine allergy.

Wider implications of the findings: Our report underlines the possibility to utilize indocyanine green (ICG)-enhanced fluorescence to localize the ureter during complex surgery for DIE, even in cases with atypical anatomy of the lower urinary tract.

Trial registration number: not applicable

POSTER VIEWING
ETHICS AND LAW

P-351 Ethical challenges posed by an increase of surplus frozen embryos in Argentinean fertility centers

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Study question: Can an increase in the quantity of frozen embryos lead to more difficulties in embryo disposition decisions (EDD)?
Summary answer: EDD posed clinical and ethical challenges and might be influenced by having more available embryos, due to changes in the laboratory procedures.
What is known already: Previous research suggests that many people find EDD difficult and emotionally distressing. Patients face ambivalence during the decision-making process which could lead to embryo abandonment. Regulation of embryo dispositions varies among countries, but in the Latin American context, the regulatory gap generates insecurities in healthcare professionals. Cultural values towards the embryo can be associated with discomfort, guilt, or psychological burden. Studies suggest that patients often feel that they are unable to make a satisfactory decision when presented with the current embryo disposition options. Thus, other 'solutions', such as the request for nonreproductive transfer, appear and raises ethical questions and concerns.
Study design, size, duration: This is an observational study which follows a thematic literature review, that identifies the main reasons for difficulties with embryo disposition decisions, in different countries. It focuses on the regulatory background of the principal ART providers worldwide to discuss the best course of action for Argentina, that faces the problem of EDD at a regulatory level. To inform the discussion, a comparative survey from an Argentinean context, was carried out.
Participants/materials, setting, methods: Most fertility clinics in Argentina are private entities, as there are very few public providers. Access to ART treatments has been regulated since 2013, but the law fails to define a number of important issues, including EDD and national registries. An online survey was sent to all reproductive facilities to collect data on storage content and the results were complemented with data from the Latin American Register (RedLara) and the Argentine Registry of Assisted Fertilization.
Main results and the role of chance: The survey results showed that in 2017, there were approximately 54.432 frozen embryos stored in 46 Argentinean fertility centers and the total amount in 2020 reached 91.724 stored in 54 centers. Despite the number of treatment cycles (IVF + OD) being constant between 2017 and 2020 (with a slight increase of 8%), the number of frozen embryos has increased exponentially (by 68.5%). This is a consequence of the improvements in cryopreservation techniques (vitrification) and the development of more efficient ovarian stimulation protocols, that have facilitated a rise in elective single embryo transfer. These advances, coupled with an inefficient regulatory framework, generate uncertainties in physicians who might already be conflicted and therefore provide little or inadequate guidance for patients facing EDD. Three strategies could be implemented to facilitate EDD under this particular setting. First, counseling sessions at different treatment stages should be encouraged and are conducted by trained mental health professionals, who are aware of their patient's changing attitudes towards surplus embryos. Second, both aneuploid embryos and embryos which were cryopreserved more than 10 years ago could form part of a national bank for research purposes, using classified storage content. Third, promote effective regulation that includes EDD and explicit storage limits.
Limitations, reasons for caution: The influence of the Catholic Church on policy makers regarding embryo dispositions is the main drawback. There is a need to foster a regulatory framework that considers the changes in IVF procedures and practices. On a practical level, psychosocial care is missing as part of healthcare teams' practices.
Wider implications of the findings: The survey results revealed that IVF centers in Argentina will face an increase in euploid, aneuploid and untested frozen embryos, due to the changes registered in laboratory procedures. This tendency shows the need to discuss EDD with patients from the beginning of fertility treatment, through to its conclusion.
Trial registration number: not applicable

P-352 Beyond individualisation: towards a more contextualised understanding of women's social egg freezing experiences

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Study question: What are the moral perceptions and views of women considering social egg freezing?
Summary answer: Participants did not perceive egg freezing as a morally problematic solution to societal problems but addressed concerns about relationship formation and wanted more social efforts.
What is known already: Central to the social egg freezing debate is the individualisation argument which underlines the idea that it is morally problematic to use individual medical-technological solutions, such as egg freezing, to solve the societal challenges women face, for instance in the current labour market. It has been said that, instead of quick medical-technical solutions that target individual women's bodies, we should focus on substantive changes that target the androcentric work culture. This theme relates to feminist concerns about unnecessary medicalisation geared towards women. Furthermore, there is a call for more empirical studies to back up this central normative claim.
Study design, size, duration: Seventeen participants were recruited by psychologists working in two Belgian centres for reproductive medicine which offer egg freezing for social reasons. In addition, four participants were recruited through via social networks. Interviews took place between February 2019 and November 2020 at a location of the participants' preference or through online video connections.
Participants/materials, setting, methods: At the beginning of the interview, open questions were asked to invite the participants to speak about social egg freezing in their own words. In the second part of the interview, we used four cards with controversial statements based on a study of the bioethics literature, to encourage the participants to reflect about ethical concerns. In this part, we engaged in Socratic dialogue. For the analysis, thematic analysis was used combined with interdisciplinary collaborative auditing.
Main results and the role of chance: This is the first study providing empirical evidence about (potential) egg freezers' moral reasoning about individualisation arguments. Most participants in our study could make sense of the individualisation argument but emphasised another societal challenge rather than the current labour market. They highlighted 'the lack of a partner relationship' as driving their motivation for this procedure. The shortage of eligible partners has been well defined in social science scholarship about social egg freezing but this element has rarely been articulated in the premises of individualisation arguments. This topic of relationships is challenging to analyse from a normative perspective because it was experienced as much more personal and intimate by the women in our study than for instance measures to realise more fair labour conditions, such as improved access to childcare. Some participants believed egg freezing resulted from individual problems and found the individualisation argument not applicable to their own situation. Furthermore, no participant found the individualisation argument legitimate to depict social freezing as morally problematic. Nonetheless, the participants showed a sense of sympathy with women who lack access to egg freezing and were in favour of societal solutions in several public domains.
Limitations, reasons for caution: Given that we report on a small-scale qualitative study of possible social egg freezers at two Belgian fertility clinics, and that our study foregrounds the voices of mostly white higher educated women who were able to afford this technology, our results cannot be generalised to all social egg freezers.
Wider implications of the findings: Our findings can contribute to a better understanding of previously identified normative arguments (e.g., individualisation and unnecessary medicalisation). There is a definite need to further analyse the complex interplay between respecting autonomous choices and evaluating contextual factors in this debate and other practices where similar individualisation arguments are used.
Trial registration number: Not applicable

P-353 When Parents and Minor Children Disagree about Fertility Preservation: A Scoping Review and Ethical Analysis

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Study question: Periodically, parents and children disagree about whether to pursue fertility preservation (FP). How should medical teams navigate these ethically complex situations?
Summary answer: Several considerations must be weighed, including the minor's age, the burden of the proposed procedure, and whether the minor or parent seeks to decline FP.
What is known already: As reproductive technology advances, FP prior to gonadotoxic therapy has become the standard of care. Periodically, parents and children disagree about whether to pursue FP. To date, there is no clear guidance on how to navigate these difficult situations. Prior studies have demonstrated that adolescents undergoing gonadotoxic therapy want their views regarding FP to be taken into account, and also that most children and adolescents are comfortable with parental involvement in decision-making. However, transgender adolescents pursue FP at lower rates than adolescents with cancer, and more research is required to elucidate the unique needs and barriers of transgender youth.
Study design, size, duration: This study involves a scoping review and ethical analysis about parent-child disagreement regarding FP in minors. The review analyzes papers that either demonstrate that parent-child disagreement occurs, describe the preferences of parents or children regarding decision-making around FP, or provide recommendations that can be used to resolve parent-child conflicts. The ethical analysis weighs relevant rights and interests, including the child's best interest, the right to an open future, the child's autonomy, and parental autonomy.
Participants/materials, setting, methods: A search string was developed to identify all relevant published manuscripts on the topic of FP in minors, including studies on decision-making, family relations and ethical challenges. The search was run through several databases, abstracts were screened using Covidence, and data were extracted from full texts. Data abstracted from the review and existing literature on general medical decision-making for minors were used to construct an ethical framework for parent-child disagreements regarding FP in minors.
Main results and the role of chance: Published work directly on the topic of parent-child disputes regarding FP is limited, however a number of studies tangentially discuss parent-child disagreements and provide insight into the desires of parents and children regarding decision-making around FP. Studies suggest that adolescents desire to have their views taken into account, and a minority of adolescents believe their wishes alone should be followed. The age of the minor is a crucial factor, and some propose that as adolescents approach adulthood, their autonomy should increase. At the same time, in practice, legal and financial constraints often render parents the ultimate decision-makers. Our ethical analysis weighs competing considerations, including the child's best interest, the right to an open future, the child's autonomy, and parental autonomy. It concludes that who prevails should depend on contextual factors, including the minor's age, the burden of the proposed procedure, and whether the minor or parent seeks to decline FP. There may also be special considerations for transgender adolescents, some of whom might have deeply personal reasons for pursuing or forgoing FP that are not well-understood by cisgender parents.
Limitations, reasons for caution: The scoping review captured a variety of results, including survey and interview studies, society guidelines, and ethical analyses. As such, we were unable to define a uniform quality metric. However, we aimed to be more rather than less inclusive because of the limited results directly pertaining to parent-child disagreements.
Wider implications of the findings: This study provides a robust review of decision-making for FP in minors and offers an ethical framework for weighing countervailing considerations when parents and children disagree about whether to pursue FP. The conclusions can be used to inform guidance for clinicians presented with this challenging ethical dilemma.
Trial registration number: N/A

POSTER VIEWING
IMPLANTATION AND EARLY PREGNANCY

P-354 Analysis of pregnancy and miscarriage rates in anti-centromere antibodies (ACA)-positive patients treated with ART

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Study question: Do ACA have an effect on pregnancy and miscarriage rates of human embryos?
Summary answer: The present results suggest that in ACA-positive cases, the pregnancy rate per transfer was significantly lower, although the miscarriage rate was not affected.
What is known already: We have previously shown that patients with high levels of anti-centromere antibody (ACA), (one of the anti-nuclear antibodies (ANA)), frequently have dispersal of the female chromosomes in the cytoplasm. Additionally, we reported that the clinical outcome was characterized by a low oocyte maturation rate following ovum pick up and high multiple pronuclear formation rate after fertilization. However, the post-implantation course of embryos with ACA-positive cases has not yet been reported. Therefore, in this study, we analyzed the pregnancy and miscarriage rates in ACA-positive patients treated with Assisted Reproductive Technologies (ART).
Study design, size, duration: 6581 patients who underwent embryo transfer after antinuclear antibody testing between January 2014 and February 2020 were included in the analysis.
Participants/materials, setting, methods: The subjects were classified into three groups: ANA-negative (without ACA or any other ANA), ACA-positive (with only ACA) and ANA-positive (with ANA but not ACA). The cycle in which the gestational sac was confirmed was considered a positive pregnancy. The pregnancy and miscarriage rates were compared among the groups using "Ryan Test" for statistical analysis.
Main results and the role of chance: Of the 6581 eligible cases, the incidence of antinuclear antibody were 71.3% (4695/6581; ANA-negative), 0.9% (61/6581; ACA-positive) and 27.7% (1825/6581; ANA-positive). The pregnancy rates based on the total number of embryo transfer cycles for each were ANA-negative: 31.5% (5283/16792), ACA-positive: 17.6% (41/233), and ANA-positive: 32.4% (1891/5833). The pregnancy rates were significantly lower in the ACA-positive group than in the other groups. The miscarriage rate was 29.4% (1553/5283) in ANA-negative, 31.7% (13/41) in ACA-positive, and 28.0% (529/1891) in ANA-positive, with no significant difference between the three groups.
Limitations, reasons for caution: Retrospective analysis
Wider implications of the findings: ACA-positive patients may benefit from a treatment strategy to increase the absolute number of oocytes by obtained in order to increase the chances of normal fertilization and attainment of implantation.
Trial registration number: none

P-355 Cancer diagnosis among patients with recurrent pregnancy loss: a cohort study

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Study question: Is unexplained recurrent pregnancy loss (RPL) related to long term cancer morbidity?
Summary answer: Recurrent unexplained pregnancy loss patients showed lower cancer morbidity. This trend was significant in the secondary aborters and in a sub-analysis for gynecological cancers.
What is known already: The association between infertility and cancer was studied, but has scarcely been studied in RPL; One study reported a higher incidence of breast and uterine cancers, while another found no association. Immune dysfunction is a possible cause of 'unexplained RPL'; RPL patients have an increased number of toxic natural killer cells (NKs) in both peripheral blood and decidua. The immune system is also involved in the recognition of cancer cells, potentially leading to effective killing. It is possible that the NK populations in RPL are capable of a better response towards cancer cells in the uterine environment and related organs.
Study design, size, duration: A retrospective cohort study comparing RPL patients and patients with normal deliveries presenting between 1990 -2010 and followed up until 2018.
Participants/materials, setting, methods: The RPL (exposed) group consisted of patients with 3 or more losses between 5-24 weeks. The comparison (unexposed) group included women who gave birth, and were not listed in the registry of RPL patients. Matching was based on maternal age and year of delivery, which was matched to the date of admission to the RPL clinic. Patients' data were cross-linked to the national cancer registry. Kaplan-Meier survival curves were used to compare cancer incidence.
Main results and the role of chance: The RPL group comprised of 937 RPL patients, compared to 4685 patients with a live birth. The mean follow up time was 16.3 ±5.3 years for RPL cases and 15.9 ± 4.9 for the comparison group. Groups were compared in terms of lifetime risk, post-admission risk and according to cancer type. In a Univariate analysis, the life time risk for cancer was 5.3% (49/937) among RPL patients and 6.8% (317/4685) in the comparison group (p=0.08). Survival analysis showed the same trend - a lower cancer morbidity in RPL patients (p=0.06). The low cancer morbidity was more prominent, reaching statistical significance in secondary RPL patients (p=0.05) , but not in primary RPL (p=0.4). Breast cancer was the most common tumor, but was neither more nor less common in RPL than in the comparison group. Gynecological cancers, however, were significantly less common in RPL patients: 0.3% (3/937) compared to 1.3% (60/4685) in the comparison group (p=0.01). After adjustment for maternal age the odds ratio for gynecological cancer was 0.247 (p=0.018, 95% CI 0.077-0.791) and significantly represented in the survival analysis (p=0.01).
Limitations, reasons for caution: There was no access to BMI and smoking status. Patients were followed for a mean period of 16 years; cancer may present later than 16 years.
Wider implications of the findings: Unexplained RPL is assumed to have an immunological basis. Our study may provide an indirect support for hyper-responsive immunological mechanisms in RPL patients. Further research is needed to deepen our understanding of the underlying mechanisms and possibly to facilitate treatment options.
Trial registration number: not applicable

P-356 Oral administration of sodium tungstate to a swine model improves embryo implantation rate

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Study question: Does sodium tungstate treatment improve embryo implantation and therefore, fertility in large mammals?
Summary answer: Oral administration of sodium tungstate increases embryo implantation and reproductive efficiency in large mammals.
What is known already: Sodium tungstate (ST) has shown its capacity to modulate critical molecules in the embryo implantation process. ST showed a positive effect on PCOS-like model to restore ovulation and fertility. Moreover, ST proved to act directly on the endometrium to increase embryo adhesion in *in vitro* assays. There is an inherent difficulty in studying implantation using *in vivo* models due to the close communication between ovary, embryo and endometrium. For the current study, the Large-White swine breed has been selected because of its high efficiency in ovulatory and fertilisation processes, minimising low embryo quality interferences in the implantation process.
Study design, size, duration: A randomised, blinded, prospective, placebo-controlled study was performed to evaluate ST effect on fertility, ovulation, and embryo implantation rates in swine, which is characterised by a high fertilisation rate but a limiting implantation rate. Forty-four primiparous Large-White sows (8 months old) were orally-treated with ST or placebo for 44-46 days, from 10 days prior to starting a progestin-based treatment for ovulation induction to gestational days 11th-13th (i.e., the window of implantation in swine). Participants/materials, setting, methods: Animals were randomised in treatment groups based on body weight ranges and housed individually in temperature-controlled conditions. 2.5g ST (diluted in 5ml of distilled water) or vehicle were once-daily orally administered with a syringe. Sows responding to ovulation-induction protocols were inseminated with high-quality sperm from untreated pigs and euthanised at gestational days 28-30 (1st pregnancy trimester) to recover genital tracts. Pregnancy, number of ovulations, number of viable/non-viable implanted embryos and fetal measurements were immediately recorded.
Main results and the role of chance: All 44 sows involved in the study responded to ovulation induction and were inseminated, but 4 females were excluded from the study because of uterine anatomical abnormalities (unicornuate uterus) or abnormalities during pregnancy. Hence, 19 ST-treated and 21 placebo sows were eligible.There were no differences in pregnancy rate (pregnancy was observed in 17 ST-treated sows 19 placebo-treated sows; 89.47% and 90.48%, respectively) or number of ovulations (21.5±4.1 vs 21.8±2.9 in placebo and treated animals, respectively; p=0.300). However, implantation rate was significantly improved in ST-treated animals, since the number of implanted embryo was found to be increased by 15% per sow in the ST-treated group; which means two additional good-quality embryos per sow (16.5±3.2 in the ST group vs 14.4±3.9 in the placebo group, p<0.05). The percentage of viable implantations, calculated as the number of viable embryos divided by the total number of viable and non-viable implanted embryos was also increased by the ST treatment (91.6±7.9 vs 96.2±4.7 in treated vs placebo groups, p<0,05). Finally, there were no effects of the treatment on the foetal phenotype, body mass and size.
Limitations, reasons for caution: The current study is the first attempt to evaluate ST effect on reproductive outcomes, in healthy large mammals. Having in mind that the selected model is high reproductive efficient, further studies assessing ST effects in infertile and sub-fertile mammals should be performed to elucidate ST activity in suboptimal fertility conditions.
Wider implications of the findings: Sodium tungstate treatment proves, for the first time, the improvement of fertility in healthy large mammals. Sodium tungstate treatment improves endometrial implantation and therefore, fertility efficiency. Thus, after subsequent further research, sodium tungstate may become a potential treatment for improving embryo implantation, an unmet medical need.
Trial registration number: not applicable

P-357 The risk factors for early pregnancy loss based on a logistic model following 13,977 infertile patients after in vitro fertilization

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Study question: What are the risk factors for early pregnancy loss (EPL) after in vitro fertilization-embryo transfer (IVF-ET)?
Summary answer: The maternal age, gestational sac diameter, embryonic length, yolk sac diameter, heart rate of day 27-29 and endometrium thickness on transfer day were risk factors. What is known already: The first routine ultrasound scan is commonly arranged on day 27-29 after IVF-ET in most reproductive centers in China to determine the location and viability of the embryo. Individual maternal factors, such as a high maternal age (MA) and abnormal ultrasound parameters such as embryonic bradycardia and excessively large or small yolk sac diameter (YSD) have been shown to be associated with pregnancy failures. However, few studies focused on the risk factors of the IVF population, and little is known about the clinical meaning of ultrasound indicators of 27-29 days after transplantation.
Study design, size, duration: This was a retrospective study in a single reproductive centre. The infertile patients included in this study underwent IVF

treatment between June 2016 to December 2017. Participants/materials, setting, methods: During this period, 13,977 women were identified with a singleton pregnancy by TVS at day 27-29 after IVF-ET. The gestational sac diameter (GSD), embryonic length (EL), embryonic heart rate (EHR) and YSD and the presence of intrauterine hematoma (IUH) were measured. The clinical characteristics were also collected. The first trimester pregnancy outcome of these women was noted at 12 weeks of gestation. A backward Wald logistic regression model was established to screen the risk factors.

Main results and the role of chance: 1,926 cases of spontaneous miscarriage ≤12 weeks of gestation, which were assigned as EPL and 12,051 women with an ongoing pregnancy for >12 weeks of gestation.

When compared with the ongoing pregnancy group, the MA, infertility duration and transfer cycle were significantly higher, and the day-14 human chorionic gonadotrophin and the endometrium (EM) thickness on transfer day were significantly lower in the EPL group ($p < 0.001$). Based on the TVS measurements, the GSD (18.5±3.6 vs. 13.2±4.8 mm), EL (3.5±0.9 vs. 1.2±1.6 mm), YSD (3.6±0.4 vs. 2.6±1.5 mm) and EHR (114.5±12.2 vs. 42.4±53.5 bpm) were significantly greater in the ongoing pregnancy group than those in the EPL group ($p < 0.001$). The incidence of IUH (16.0% vs. 18.8%, $P = 0.002$) was also markedly higher in the EPL group

MA, GSD, EL, YSD, EHR and EM on transfer day finally entered the logistic model after stepwise screening. The probability of EPL was: $\exp(z)/(1 + \exp(z))$, where $z = 2.432 + (0.092 \times MA) - (0.074 \times EM) - (0.114 \times GSD) - (0.245 \times EL) - (0.034 \times HR) - (0.159 \times YSD)$.

Limitations, reasons for caution: Data on smoking and clinical symptoms such as vaginal bleeding or abdominal pain were not included in the final analysis which might be possible risk factors. These predictors were derived from an IVF population, the situation may not be the same in the general population.

Wider implications of the findings: The risk factors for EPL after IVF-ET are clearly identified in this study. The logistic model which incorporates readily available data that are routinely collected in clinical practice could be used for calculating the risk of EPL and effectively guide subsequent medical plans.

Trial registration number: None

P-358 Characteristics of patients with inherited thrombophilia and anticoagulant treatment in repeated implantation failure (RIF) and recurrent pregnancy loss (RPL)

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Study question: Do patients with inherited thrombophilia associated to RIF and RPL benefit from anticoagulant therapy?

Summary answer: Low molecular weight heparin (LMWH) in patients with medium and high risk of hereditary thrombophilia, associated with RIF could improve the reproductive prognosis.

What is known already: Thrombophilia is a condition that can be acquired and/or inherited genetically, that is characterized by the predisposition of patients to form venous and arterial thromboembolic events. Inherited thrombophilia has been associated with different complications during pregnancy, such as RPL. Genetic variants linked to hereditary thrombophilia can be classified by the thromboembolic risk: low (F12, F13A1, FGB), medium (MTHFR, PROCR, PROS1, SERPINC1, SERPINC1 PAI-1) and high (F2, F5, GP1BA), according to Martinez - Zamora. RPL rate may reduce with anticoagulant therapy. However, there is no conclusive evidence that prophylactic treatment improves the pregnancy rate in infertile women during IVF.

Study design, size, duration: We performed a prospective observational study which included patients referred to Ceras Clinic between March 2018 and March 2020, due to RPL (n=38) and RIF (n=40). All patients underwent genetic analysis for hereditary thrombophilia(F13, F2, F5, FGB, GP1BA, MTHFR C677T, MTHFR A1298C, PAI1,, PROCR, SERPIN1 CM910058, SERPIN 1 CM920113 ,F12, PROS1) by Sanger sequencing. The characteristics of anticoagulant therapy with clinical pregnancy rate and LBR were analyzed, using chi-squared test with STATA version 16.

Participants/materials, setting, methods: Patients have been included in the study according to their past medical history (stroke or myocardial infarction, personal or familiar history of deep vein thrombosis or pulmonary embolism, smoking, hormone replacement therapy), and reproductive history. Two groups were formed, the first group (n=40) corresponds to RIF, and the second (n=38), RPL. Genetic study of hereditary thrombophilia (11 genes) was performed to examine the genetic risk and assess the administration of anticoagulant therapy.

Main results and the role of chance: The prevalence of pathological antecedents in patients with RIF and RPL was not statistically significant (p>0,05), indicating that the factors that contribute to poor reproductive outcomes in these two groups of patients could be similar. Patients with RIF had a medium risk of thrombophilia in 65%, followed by low risk in 32.5% and high risk in 2.5%. RPL group presented 78.95%, 15.79% and 5.26%, respectively. All patients with medium and high risk for thrombophilia received anticoagulation. Clinical pregnancy rate (69.7%) and live birth rate (63.64%) were not statistically significant (p> 0.05) in RPL with anticoagulant therapy, compared to RPL group who did not received treatment (clinical pregnancy rate and live birth rate in 60%). Therefore, it is proposed that there may be other factors associated with abortions that require investigation. However, clinical pregnancy rate (77.14%) and live birth rate (74.29%) were statistically significant ($p < 0.05$) in RIF with anticoagulant therapy, compared to RIF group that did not received treatment (clinical pregnancy rate and live birth rate in 20%). This suggests that there could be a beneficial factor due to anticoagulation. Further studies are needed to assess that anticoagulant treatment could improve obstetric outcomes in patients with RIF and RPL.

Limitations, reasons for caution: The small number of patients assessed is the main limitation of this work. Larger studies must be designed to accurately determine participation of each mutation associated with recurrent implantation failure and recurrent pregnancy loss. The role of anticoagulant therapy should be evaluated in randomized clinical trials.

Wider implications of the findings: Establishing a stronger evidence base implies that future studies should include large population groups. It is primordial to assess whether it is cost-effective to determine the risk of inherited thrombophilia in RIL and RPL, to increase the live birth rate by anticoagulant therapy. The information is controversial to this day.

Trial registration number: 'not applicable'

P-359 Blastocyst quality, transfer difficulty and endometrial thickness affect clinical pregnancy after frozen embryo transfer (FET) of euploid blastocysts in the upper uterine cavity

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Study question: Which factors affect the clinical pregnancy rate (CPR) after single euploid frozen embryo transfers (FET), when the blastocyst is transferred in the upper uterine cavity area?

Summary answer: Blastocyst quality, embryo transfer difficulty and endometrial thickness affect the CPR in FET.

What is known already: There is a limited understanding of the factors affecting success rates after FET. The most important factors influencing implantation rates are patient characteristics, type of endometrial preparation, embryo quality and transfer difficulty. It has been shown that the position of the euploid blastocyst, measured as distance from the fundus (DFF) of the uterine cavity (mm), affects the implantation potential. Although the ideal location within the uterine cavity is still being debated in very heterogeneous patient populations, most studies have found that the highest pregnancy rates are obtained when the embryo is placed in the upper area of the uterine cavity.

Study design, size, duration: This single center retrospective cohort study included a total of 603 single euploid FET cycles, in the upper half of the uterine cavity, between January 2019 and November 2020 in ART Fertility Clinic Abu Dhabi, UAE.

Participants/materials, setting, methods: Trophectoderm biopsy samples were subjected to Next Generation Sequencing to screen the ploidy state. Vitrification and warming were performed using the Cryotop method (Kitazato, Biopharma). The full length of the uterine cavity and the longitudinal distance between the fundal endometrial surface and the air bubble after transfer were measured.

Main results and the role of chance: The patients were on average 33.9 (19-46) years old. The FET was performed in a natural cycle (NC) (n=278) or

hormone replacement therapy (HRT) (n=325). Of the 603 transfers which had been performed in the upper half of the uterus, 412 (68.3%) resulted in a pregnancy and 311 (51.5%) in a clinical pregnancy. After bivariate analysis, the clinical pregnancy rate was significantly higher for high quality blastocysts (grade 1-2 versus 3-4) (p<0.001), after easy embryo transfers (p=0.001) and for higher endometrial thickness (p=0.027).

After performing a multivariate logistic regression analysis to consider the effect of all explanatory variables (age, Anti Müllerian hormone, body mass index, endometrial thickness, quality of the blastocyst, difficulty of the transfer [requirement of additional instrumentation], presence of mucus or blood on the transfer catheter, day 5 or day 6 biopsy, FET endometrial preparation), the clinical pregnancy was affected by the endometrial thickness: OR 1.20 [1.05-1.37], p=0.007; transfer difficulty: OR 0.44 [0.25-0.79], p=0.006; blastocyst quality 3: OR 0.38 [0.18-0.79], p=0.01 and blastocyst quality 4: OR 0.15 [0.06-0.37], p<0.0001. Age did not affect the clinical pregnancy after transferring a single euploid blastocyst: OR 1.03 [1.00-1.06], p=0.052.

Limitations, reasons for caution: The limitation of this study was its retrospective nature and the small sample size. Other parameters may be important in live birth outcomes.

Wider implications of the findings: Optimization of clinical pregnancy outcomes after FET depends on multiple factors. Even after transfer of euploid blastocysts in the upper uterine cavity, the endometrial thickness, transfer difficulty and blastocyst quality will still affect the clinical pregnancy outcomes.

Trial registration number: NA

P-360 Blastocyst biopsy day does have an impact on clinical pregnancies in different frozen embryo transfer (FET) regimens: natural cycle (NC) versus hormone replacement therapy (HRT)

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Study question: Do euploid blastocysts biopsied on day (D) 5 or D6 differ in clinical pregnancy rates when single FET are performed in NC or HRT cycles?

Summary answer: In single FET cycles, euploid D5 blastocysts have higher clinical pregnancy rates than D6 in NC, while outcomes are comparable in HRT cycles. What is known already: The synchronization between the endometrium and the embryo development is fundamental for a successful implantation. When performing FET with euploid blastocysts biopsied on D5 or D6, higher clinical pregnancy rates have been reported with D5 blastocysts, however contradictory findings were described due to the study design heterogeneity and endometrial preparation (EP) protocol variabilities. In FET cycles, no consensus has been defined of the superiority of NC over HRT cycles when euploid blastocysts are transferred. Consequently, the question remains unanswered if the clinical pregnancy rates of single euploid FET with D5 or D6 blastocysts differ when the EP protocol remains constant.

Study design, size, duration: A single center observational study was performed between June 2017 and November 2020, including 1027 single euploid FET with blastocysts biopsied on D5 or D6. All patients with primary or secondary infertility who underwent a FET in a NC or HRT EP protocol, with blastocysts graded ≥ BL3CC (Gardner scoring system) prior to biopsy were included. Vitrified-warmed blastocysts that did not re-expand within 1-hour post-warming were excluded from the analysis.

Participants/materials, setting, methods: In NCs, vaginal progesterone (P4) (Endometrin®) was administrated (3x100mg) after endocrinological confirmation of ovulation until pregnancy test. For HRT cycles, oral estradiol administration was started on day 2 (4 mg) and increased to 6mg on D5 of the cycle. When endometrial thickness was ≥6 mm, P4 was given (3x100mg) until pregnancy test. All FET were performed on D5 after start of P4 administration. Clinical pregnancy was recorded as the presence of an intrauterine gestational sac.

Main results and the role of chance: Women's mean age was 33.8 ± 5.5 years. A total of 651 FETs were performed with D5 euploid blastocysts (37.6% in NC and 62.4% in HRT) and 376 with D6 (43.1% in NC and 56.9% in HRT). Clinical pregnancy rate in NC was higher with D5 blastocysts compared to D6 (66.9% vs 50.0%; OR=0.494, 95% CI=0.322-0.758; p<0.001), while no significant differences were found when vitrified-warmed blastocysts were transferred in HRT cycles (64.3% vs 58.4%; OR=0.781, 95% CI=0.548-1.112; p=0.164). Additionally, clinical miscarriage was significantly higher with D5 euploid blastocysts transferred in NC (D5=10.9% vs D6=3.7%, OR=0.239, 95% CI=0.044-0.837; p=0.019). In HRT, miscarriage outcomes were similar between D5 and D6 euploid blastocysts (D5=18.7% vs D6=20.8%, OR=0.781, 95% CI=0.548-1.112; p=0.164), but significantly higher (p<0.001) than in NC. From a multinomial logistic regression model including age, blastocyst quality and day of biopsy as confounding factors, the clinical pregnancy rate was significantly affected by D6 blastocyst biopsy (OR=0.571, 95% CI=0.360-0.906, p=0.017) and inner cell mass (ICM) grade A (OR=3.941, 95% CI=1.149-10.402; p=0.006) or B (OR=2.601, 95% CI=1.146-5.907, p=0.022) in NC. In HRT cycles, exclusively ICM was statistically significant (OR=2.555, 95% CI=1.214-5.381, p=0.015 and OR=2.397, 95% CI=1.286-4.470, p<0.001 for grade A and B, respectively).

Limitations, reasons for caution: The current results are based on an observational retrospective study. Live birth and perinatal outcomes should be considered in a further analysis to evaluate the performance of the NC vs HRT protocols when D5 or D6 euploid blastocysts are transferred in FET cycles.

Wider implications of the findings: While the clinical pregnancies of D5 and D6 euploid blastocysts are comparable in HRT protocols only, the miscarriage rates seem to be significantly increased as compared to NC. Further studies are required to personalize EP protocols based on the day of blastocyst biopsy in order to improve clinical outcomes.

Trial registration number: No

P-361 Endometrium optical coherence tomography (OCT) and histomorphometry in implantation window and their relationships with reproductive failure and implantation outcome

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Study question: How do endometrium OCT image characteristics during peri-implantation window correlate with histomorphometry and associate with implantation outcomes in women with reproductive failure?

Summary answer: Endometrium OCT intensity correlated with stromal cell density and gland size. Endometrium with recurrent implantation failure had low OCT intensity but reversed in successful implantation.

What is known already: OCT is a non-invasive imaging technique using low energy near-infrared light to capture micrometer-scale resolution images from optical scattering media. An image produced by OCT resembles tissue architecture observed in histology, so OCT imaging has been regarded as "optical biopsy". Our previous findings demonstrated OCT is better than ultrasound to identify endometrial pathology. We also showed association of OCT signal with microvessel density in peri-implantation endometrium. However, other histomorphometry were not evaluated. It is still unclear whether endometrium OCT image characteristics are different in reproductive failure and can predict implantation outcomes.

Study design, size, duration: This was a prospective study conducted at teaching hospital of The Chinese University of Hong Kong from Jan 2018 to Dec 2019. 46 infertile women with or without recurrent miscarriage (RM) and implantation failure (RIF) were recruited in this study. Endometrium OCT imaging and subsequent biopsy were performed on the seventh day after luteal hormone surge (LH+7) in natural cycle prior to the consecutive natural conception or embryo transfer (ET) cycle.

Participants/materials, setting, methods: At least 5 systematic random endometrium OCT images from uterine fundus, body and lower segment of each subject were included for intensity analysis by two independent observers. OCT intensity of each image was classified as low, moderate, high based on optical range and then average OCT intensity in each uterine region was calculated for group comparison. Endometrium glandular epithelial, stromal,

endothelial, uNK cells were defined by standard H&E and specific immunostaining for histomorphometry and correlation.
Main results and the role of chance: OCT intensity significantly correlated with endometrial cell and gland parameters regardless classifications of reproductive failure and implantation outcome. Higher OCT intensity indicated higher stromal cell density, gland to stromal (G/S) ratio and average gland area, but fewer microvessel and uNK cells. None of the endometrium histomorphometry were significantly different among different reproductive failure types and implantation outcomes, suggesting it may not be sensitive enough to detect the abnormal histological features. However, OCT intensity was significantly lower in the uterine fundus and body of RIF group than in that of infertile and RM groups. There was no significant difference of OCT intensity in the lower part of the endometrium among three groups. It indicates that OCT intensity is a sensitive marker to differentiate endometrium with RIF from the endometrium with other conditions and also endometrium with RIF is characterized with less stromal cells and smaller glands. Compared with infertile group with unsuccessful implantation, OCT intensity was higher in all three parts of the uterus from the infertile group with successful implantation, but the results were not statistically different. The results further implied that endometrial cells and gland size may potentially contribute to the endometrium receptivity for implantation.
Limitations, reasons for caution: Current endometrium OCT imaging depth is within 3mm, change beyond this thickness is inaccessible but still the most important layer for implantation. This is a pilot and small study with lack of normal fertile control. Endometrium OCT imaging in the same natural conception or ET cycle will be more accurate.
Wider implications of the findings: OCT imaging could be used as a potential noninvasive modality to evaluate peri-implantation window endometrium. It enables real-time and in-situ visualization of endometrium structure and pathology with no additional biopsy risk and examination delay. Larger clinical trials are needed to confirm its clinical applications and utilities.
Trial registration number: CREC 2016.160

P-362 The effect of nolasiban on uterine contractility at the time of embryo transfer in in vitro fertilisation patients

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Study question: What is the effect of nolasiban on the uterine contractility of in-vitro fertilisation (IVF) patients prior to embryo transfer (ET) ?
Summary answer: A single oral dose of nolasiban 900 mg administered 4 h before ET significantly decreased contraction frequency and increased coordination compared to placebo.
What is known already: Nolasiban is an investigational oral oxytocin receptor antagonist (OTRa) being developed to improve the chance of pregnancy following ET. Increased uterine contraction frequency can influence embryo implantation, and the coordination of these uterine contractions is equally important. OTRa have been shown to decrease uterine contractions and increase endometrial perfusion. Recently, an automated and quantitative measurement tool using transvaginal ultrasound (TVUS) to better characterise uterine contractility has been developed which can be used to quantify the effect of nolasiban on uterine contractility.
Study design, size, duration: This study is part of a completed multi-centre randomised placebo-controlled trial (IMPLANT 1 – NCT02310802) in IVF patients (n=247) carried out in 2015. Our study retrospectively assessed a subset of patients with good quality TVUS recordings to evaluate their mechanical uterine motion that were randomised to receive either nolasiban 900mg (n=39) or placebo (n=42).
Participants/materials, setting, methods: Subjects were < 37 years, undergoing ET on Day 3 following IVF/ICSI and with evidence of uterine contractions 4 h before ET. Nolasiban was administered 4 h before ET. Patients underwent TVUS immediately before drug administration and again immediately before ET. Uterine contraction frequency, amplitude, power and coordination were measured by applying dedicated speckle tracking and strain analysis. The Shapiro–Wilk test, followed by the Wilcoxon rank-sum test were applied to compare features between treatment groups.
Main results and the role of chance: The mean (SD) frequency of uterine contractions was 1.54 (0.25) in the nolasiban group versus 1.57 (0.12) in the placebo group (p = 0.016). The mean (SD) coordination was 0.10 (0.17) in the nolasiban group versus 0.02 (0.16) in the placebo group (p = 0.034). The coordination feature was measured by assessing the presence of simultaneous movements of the anterior and posterior uterine walls, a higher value reflects increased coordination. There was no difference in contraction amplitude or power.
Limitations, reasons for caution: This was a retrospective analysis of TVUS videos. The small sample size limits the generalisability of the findings. Furthermore, our initial results do not show how the changes in uterine motion may affect pregnancy rate after ET, meaning that the clinical relevance of our results remains to be proven.
Wider implications of the findings: Our results show that in patients taking one 900mg dose of nolasiban prior to ET, beneficial uterine contractions are seen, which could be promising for embryo implantation and pregnancy in IVF patients. Our quantitative TVUS measurement tool can be applied to different patient populations to accurately quantify uterine motion.
Trial registration number: NCT02310802

P-363 Poor Ovarian Response is Associated with Anti-ovarian Antibody, and Pro-inflammatory Immune Responses in Women Underwent Assisted Reproductive Technology Cycles

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Study question: To investigate if the anti-ovarian antibody (AOA) is associated with poor ovarian response (POR) and pro-inflammatory immune responses in women undergoing assisted reproductive technology (ART) cycles.
Summary answer: The POR patients have a higher prevalence of AOAs. Women with autoimmune POR (POR(+)/AOA(+)) have dysregulated pro-inflammatory immune responses and metabolic factors.
What is known already: It has been proved that AOAs play important role in diseases that related to human reproduction such as premature ovarian failure (POF) which also termed as premature ovarian insufficiency (POI), infertility, polycystic ovary syndrome (PCOS), in vitro fertilization (IVF) implantation failure, and in poor ovarian response in IVF stimulation. The POR women had elevated inflammatory immune responses: increased NK cell count and cytotoxicity, B cell counts, Th1/Th2 ratio and elevated metabolic factors such as higher homocysteine and plasminogen activator inhibitor-1 (PAI-1) level.
Study design, size, duration: This study is a retrospective cohort study between December 2015 and February 2019. 248 women who underwent ART cycles were included. Study patients were divided into four groups based on AOA test and POR diagnose defined by the European Society of Human Reproduction and Embryology consensus: POR(-)/AOA (-) group (N=148), POR(+)/AOA(-) group (N=34), POR (-)/AOA (+) group (N=44), POR(+)/AOA(+) group (N=22). Peripheral blood was collected during the early follicular phase when they enter the program.
Participants/materials, setting, methods: The natural killer (NK) cell levels and cytotoxicity, T helper (Th) 1/Th2 cell ratios were measured by flow-cytometry. Anti-phospholipid Antibodies (APA) was tested by enzyme linked immunosorbent assay (ELISA). AOA, 25 (OH) vitamin D level, homocysteine, PAI-1 level was tested by Immunofluorescence Assay.One way ANOVA was applied to compare the continuous variables among study groups. Chi-squared analysis or Fisher's exact test were performed to compare the categorical variables.
Main results and the role of chance: The POR patients have a significantly higher prevalence of AOA than non-POR patients (39.3% vs. 22.9%, P=0.017, OR 2.176 95% CI 1.156-4.099). Peripheral blood CD56+ NK cell level (%), NK cytotoxicity, CD19+CD5+ B-1 cell level (%) and IFN-g/IL-10 producing Th1/Th2 cell ratios were significantly higher in POR(+)/AOA(+) group than those of other groups (P<0.05, P<0.05, P<0.05, P<0.05, respectively). TNF-a/IL-10

producing Th1/Th2 cell ratio of POR(+)/AOA (+) group was significantly higher than those of POR(+)/AOA(-) and POR(-)/AOA(-) groups (P< 0.05, respectively). Peripheral blood homocysteine and vitamin D levels of the POR(+)/AOA (+) group were significantly lower than those of other groups (P< 0.005, respectively). Peripheral blood PAI-1 level of POR(+)/AOA(+) group was significantly higher than that of POR(-)/AOA(-) group (P< 0.05). In POR(+)/AOA(+) group, the prevalence of antiphospholipid antibody was significantly higher than that of POR(+)/AOA(-) group (54.5% vs 20.5%, P=0.005, OR 4.667, 95% CI 1.532-14.216).

Limitations, reasons for caution: This was a single center study, results need to be validated across different center and study population.

Wider implications of the findings: The diagnostic and therapeutic approaches for AOA (+) autoimmune POR patients should be differentiated from those for non-autoimmune POR.

Trial registration number: not applicable

P-364 How to estimate the probability of a live birth after one or more complete IVF cycles? The development of a novel model in a single-center

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Study question: What is the probability of a live birth for an infertile couple after one or more complete cycles of in vitro fertilization (IVF)?

Summary answer: The Cox regression and Nomogram model could estimate the chance of a live birth after a complete IVF cycle effectively.

What is known already: At present, kinds of prediction models have been established for estimating the chance of having a live birth in different centers based on the characteristics of the population. But the predictive value and effectiveness of different models were different. These models were not applicable to every reproductive center.

Study design, size, duration: A retrospective cohort study was conducted in reproductive center of Shenzhen Zhongshan Urology Hospital from January 2012 to April 2015. 4413 patients who completed ovarian stimulation treatment and reached the trigger were involved. In order to verify the efficacy, we conducted stratified sampling for the whole sample according to live birth or not.70% of the patients were divided into training set (N=3089) and 30% of the patients were divided into validation set (N=1324).

Participants/materials, setting, methods: Live birth rate (LBR) and cumulative LBR (CLBR) were calculated for up to five complete IVF cycles. PH test was used for establishing a prediction model. A Cox regression and nomogram model was built on the basis of training set, and ROC curve was used to test the specificity and sensitivity of the prediction model. And then, the validation set was applied to verify the validity of the model.

Main results and the role of chance: In the fresh embryo transfer cycle, the LBR were 38.7%. In the first to fifth frozen cycle, the optimal estimate and conservative estimate CLBR were 59.95%, 65.41%, 66.35%, 66.58%, 66.61% and 56.81%, 60.84%, 61.50%, 61.66%, 61.68%, respectively. There was no difference among the characteristics data of training and validation cohorts, which indicates that stratified sampling was reasonable. Based on the results of PH test, the predictive factors of live birth were fertilization technique, infertility factor, serum progesterone level (pg/mL) and luteinizing hormone level (pg/mL) on the day initiated with gonadotropin (R=0.043, p=0.059; R=0.015, p=0.499), basal follicle-stimulating hormone (R=-0.042, p=0.069) and BMI(R=-0.035, p=0.123). We used ROC curve to test the specificity and sensitivity of the prediction model. The AUC was 0.782(p<0.01,95%CI=76.4-80.1%). Then the model was verified in the validation data. And the AUC was 0.801 (p<0.01,95%CI=77.4-82.8%). A Nomogram model was built on the basis of possible factors that might influence the live birth rate of training data. The concordance index (C-index) was 0.53. For the validation data, the C-index was 0.525.

Limitations, reasons for caution: This study was a retrospective analysis of a single-center, which was limited by sample size. Although its efficacy and specificity have been validated internally, further prospective clinical trials are needed to validate its efficacy. Wider implications of the findings: This prediction model can effectively predict the probability of infertile couples having a live birth. Further, this model can also help clinicians to make clinical decisions and provide guidance for patients.

Trial registration number: Non-clinical trials

P-365 Altered endometrial oestrogen-responsiveness and aberrant expression of cell-fate markers may contribute to the aetiology of recurrent pregnancy loss

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Study question: Do women with recurrent pregnancy loss (RPL) have an aberrant expression of oestrogen receptor-β (ERβ) and cell-fate markers during the window of implantation (WOI) endometrium?

Summary answer: Women with RPL are found to have significantly altered levels of ERβ and Ki-67 in the WOI endometrium, possibly resulting in anti-proliferative and anti-angiogenic effects.

What is known already: RPL affects 1% of all women and has been associated with altered endometrial angiogenesis and proliferation when compared with the endometrium of healthy fertile women. RPL can be subcategorised into recurrent loss of anembryonic pregnancy, fetal loss (following evidence of a fetal heartbeat) and recurrent implantation failure (RIF). ER is the only oestrogen-receptor (ER) known to be expressed in the vascular endothelium of the endometrium and is the dominant ER during the WOI. It has an important role in endometrial regeneration and is proposed to regulate the angiogenic and vascular changes that occur in embryo implantation. Study design, size, duration: This pilot case-control study took place at the Liverpool Women's Hospital and included 38 women; 29 who suffered RPL and 9 controls with proven fertility (≥2 healthy pregnancies). Of the RPL group, 9 had recurrent loss of anembryonic pregnancy, 10 had recurrent fetal loss and 10 had RIF. Endometrial samples were collected during the WOI (cycle day 22+/-2).

Participants/materials, setting, methods: To determine whether markers of endometrial cell proliferation and oestrogen-responsiveness are associated with RPL, we assessed the immuno-staining for ERβ, progesterone receptor (PR) and cell-fate marker Ki-67 in endometrial biopsies during the WOI using immunohistochemistry. A semi-quantitative immuno-staining score was used to assess the endometrial glands, stroma, luminal epithelium, perivascular and vascular endothelium compartments separately. Statistical differences between groups were calculated by non-parametric tests and significance level set at p<0.05.

Main results and the role of chance: During the WOI, the endometrial epithelium of women with RIF and recurrent anembryonic pregnancy loss showed significantly higher levels of ERβ when compared with fertile controls (p=0.01 and p=0.01, respectively). This may indicate an anti-proliferative process occurring at the site of implantation with very early pregnancy losses. In contrast, with women with recurrent fetal loss, a significantly lower level of ER was found within the vascular endothelium when compared with the fertile controls (p<0.01). This supports the theory that increased oxygen levels may compromise trophoblastic invasion, thereby leading to fetal loss. The presence of Ki-67 (a marker of proliferation) was significantly lower within the vascular endothelium of all types of RPL: recurrent anembryonic loss (p=0.02), RIF (p=0.02) and recurrent fetal loss (p<0.01). These findings suggest ineffective endometrial angiogenesis in RPL, resulting in a suboptimal endometrial microenvironment.

PR was found to be significantly reduced (p<0.01) in the perivascular area of women with RIF versus fertile controls. Since decidualisation and preparation of the endometrium for a successful implantation is controlled by critical target genes downstream of PR, this alteration in PR may be an important feature of their defective endometrial phenotype.

Limitations, reasons for caution: Samples analysed were taken from the functional endometrium and therefore the results do not reflect the basalis. The WOI was identified using history and histological appearance, rather than timing

with ovulation. Although we detected statistical significance, generalisation of the results requires further studies with larger sample size.
Wider implications of the findings: This data provides novel insight into the biological correlates of clinical types of RPL and suggests that specific alterations in the regulation of endometrial cell fate and oestrogen- responsiveness are associated with different types of RPL. This highlights possible new therapies for RPL, such as selective oestrogen receptor modulators (SERMs).
Trial registration number: Not applicable

P-366 MicroRNAs at the embryo-maternal interface have effects on endometrial cell proliferation

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Study question: Whether cell-free microRNAs are part of the embryo-maternal interactome with possible effects on processes related to implantation.
Summary answer: Specific microRNAs cause major transcriptomic changes in uterine cells and alter cellular proliferation which is pivotal for the implantation of the incoming embryo.
What is known already: A plethora of molecules present at the uterine luminal fluid including cytokines, growth factors, and adhesion proteins are involved in implantation. However little is known about the roles of extracellular microRNAs (miRNAs) at the embryo-maternal interface. MicroRNAs act mainly as gene regulators and a single miRNA can have thousands of gene targets. MiRNAs are released by blastocysts and uterine cells internalize miRNAs that are present in the extracellular environment. To date there is limited evidence on the molecular actions of these cell-free miRNAs and their effects on processes related to implantation.
Study design, size, duration: Human endometrial stromal cells (hESCs) were cultured in complete growth medium for 8 consecutive passages. A miRNA mimic experiment in 6 replications was carried out in which endometrial cells were transfected with miR-371a. Gene changes in the hESCs were studied with genome-wide microarray technology and the results were validated *in vitro* with PCR.
Participants/materials, setting, methods: The miR-371a mimic was transfected in hESCs using a Lipofectamine reagent. RNA was extracted and the samples were processed with microarray Clariom™ Human Assays using Affymetrix®. The transcriptomic profiles between transfected and control cells were compared using Partek®. Differentially expressed genes were considered significant when p-value was <0.05, false discovery rate, FDR ≤ 0.05 with Benjamini-Hochberg correction, and fold-change of >1.5 or < -1.5. Functional enrichment analysis was carried out using WebGestalt and Enrichr.
Main results and the role of chance: MiR-371a altered the expression of 4.760 genes in endometrial cells ($p<0.05$, fold-change 1.5). A total of 16 biological processes, 23 cellular components, and 24 molecular pathways were disrupted by this miRNA. WebGestalt analysis found 159 enriched categories including increase of negative cell cycle regulation, apoptosis signalling, and cycle arrest and decreased cell proliferation. Cell cycle was one of the most affected pathways in KEGG analysis with at least 54 genes dysregulated. Mammalian phenotype ontology analysis found 4.818 affected phenotypes, including decreased cell proliferation (58 genes), increased apoptosis (48 genes) and abnormal cell cycle (41 genes). Key-genes of endometrial proliferation at the window of implantation were significantly downregulated, including: CD44, PGR; IGFs, FGFs, and HAND2. Moreover, at least 25% decreased hESCs proliferation was verified *in vitro* after transfection. These negative effects of miR-371a in cell cycle could disturb implantation of the incoming embryo, since intense cellular proliferation is necessary for establishment of the implantation site.
Limitations, reasons for caution: These results are limited to miR-371a actions on human endometrial stromal cells. It is likely that miRNAs, cytokines, growth factors, and other molecules form complex regulatory networks that control uterine receptivity and embryo implantation.
Wider implications of the findings: MiRNAs are important mediators of the embryo-maternal interactome. Their actions are likely involved in implantation-related processes including inter-cellular communication, decidualization, adhesion, invasion, and establishment of the implantation site. Embryo-secreted miRNAs change the transcriptome of the neighboring endometrial cells with effects on implantation-related pathways, serving thus secretory functions.
Trial registration number: N/A

P-367 A comparison of frozen-thawed embryo transfer protocols in 3,478 frozen embryo transfers

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Study question: What frozen-thawed embryo transfer (FET) protocol is associated with the highest live birth rate (LBR)? Summary answer: Natural cycle FET (NC-FET), with or without hCG triggering are associated with higher LBR and clinical pregnancy rate (CPR) compared to artificial HRT-FET cycles.
What is known already: FET cycles (as opposed to fresh ET) are now the most frequently performed treatment in ART. There are many reasons for this including better laboratory cryopreservation techniques, increased single ET cycles, freeze-all cycles to reduce OHSS, as well as PGT-A and personalized ET. Nevertheless, there is no clear consensus on the most effective protocol.
Study design, size, duration: Retrospective cohort study with FET of cleavage (n=220) and blastocyst (n=3258) embryos thawed 2013-2018 in a single academic center. FET protocols were NC-FET (n = 182), artificial HRT-FET (n = 3159) and modified NC (mNC) with hCG triggering (n = 137). Other cycles (gonadotrophin or GnRH agonist) and women with uterine anomalies were excluded. Primary outcome was LBR. Secondary outcomes were CPR, visits per cycle and endometrial thickness. Adjustment was made for potential known confounders.
Participants/materials, setting, methods: In NC-FET, no medication was given and ET timing was by serum LH surge. In mNC-FET, hCG was given when the lead follicle reached 18mm rather than awaiting the LH surge. In artificial HRT-FET, estradiol valerate was given and once endometrial thickness reached 8mm, progesterone was added and ET was planned. Adjustment for female age at oocyte retrieval, embryo stage, embryo grade, year of freezing, year of thawing, infertility cause and endometrial thickness was performed.
Main results and the role of chance: There were no significant differences between the groups with regard to female age at oocyte retrieval, embryo stage, embryo grade, embryo number, cycle number and endometrial thickness. As expected, more women with irregular cycles were included in the artificial HRT-FET compared to NC-FET (16.1% vs. 8.2%, p=0.003) and mNC-FET (16.1% vs. 4.1%, p<0.0001). There were more visits per cycle in NC-FET and mNC-FET compared to artificial HRT_FET ($p<0.0001$). LBR was higher in the mNC-FET (38.0%) and NC-FET (31.9%) compared to artificial HRT_FET (20.2%) ($p=0.0001$ and $p=0.0003$ respectively). CPR was higher in mNC-FET compared to artificial HRT-FET (45.3% vs. 32.3%, $p=0.0002$), and in NC-FET compared to artificial HRT-FET (44.5% vs. 32.3%, $p=0.0009$). There was no significant difference in LBR or CPR between NC-FET and mNC-FET. Sub-analysis of the first FET showed similar results. Biochemical pregnancy loss and miscarriage rates were similar in all groups. The higher LBR with NC-FET and mNC-FET remained significant even after adjusting for potential confounders, (aOR 2.42, 95%CI: 1.53-3.66, $p<0.0001$).
Limitations, reasons for caution: The interpretation of the findings of this study is limited by the retrospective nature of the analysis and the potential for unmeasured confounding variables.
Wider implications of the findings: Although artificial HRT FET cycles are more common, convenient and practical for clinicians, with less visits per cycle, its use must be cautiously reconsidered in light of the potential negative effect on LBR when compared with natural cycle FET.
Trial registration number: not applicable

P-368 Dynamic metabolomic profiling during early implantation period

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Study question: To investigate the different metabolomic profiling in serum between pregnant and non-pregnant women during early implantation period.
Summary answer: Metabolomics of progesterone-related hormones enhances from ET day3 for pregnancy women compared with non-pregnancy women.
What is known already: Metabolomics is based on high-throughput analytical methods to identify and quantify metabolites. Compared to other omics study, metabolomics is the closest one to the phenotype, allowing the observation of dynamic changes in phenotype at specific timepoints. So far there is no published work about the metabolomics profile in human early implantation period. Study design, size, duration: Study design: comparative study. Size: 14 pregnancy women and 14 non-pregnancy women. duration: time-course.
Participants/materials, setting, methods: Participants: pregnancy women and unpregnancy women after embryo transfer (ET). Setting: university-based study. Methods: Peripheral blood were collected at ET day0, 3, 6 and 9. metabolomic profiling in serum by platforms of capillary electrophoresis-mass spectrometry (CE-MS) and liquid chromatography–mass spectrometry (LC-MS).
Main results and the role of chance: There were no statistical difference of the age, BMI, basal FSH level, endometrium thickness on the day of embryo transfer, distribution of primary and secondary fertility, embryo transfer cycle as well as the infertile types between the two groups. After deleting those with over 50% missing data, we finally have 310 metabolites into statistical analysis. Among the 310 metabolite, lipid metabolites account the largest percentage, nearly half of all metabolites. The second biggest class of metabolites in our data was organic acids. Combined results in repeated measurement ANOVA (RM-ANOVA) and ANOVA-simultaneous component analysis (ASCA) as well as multivariate empirical Bayes time-series analysis (MEBA), we finally found that progesterone-related hormones were the most important metabolites for the whole time-series data. Those significant metabolites showed a significant down regulation from ET day0 to ET day3 and up regulation from ET day3 to ET day9.
Limitations, reasons for caution: we have limited sample size for this study and further validation is necessary for confirmation.
Wider implications of the findings: The phenomenon of upregulation of progesterone-related hormones from day3 in pregnancy group might be related to the embryo-originated hcg. Because the embryo has entered into endometrium at day3 and produced cytokines, hcg and other interaction with endometrium.
Trial registration number: NA

P-369 Three-dimensional co-culture of human endometrial cells with aneuploid and euploid embryos

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Study question: Is implantation different in euploid and aneuploid embryos?
Summary answer: By simulating the human endometrium using a three-dimensional scaffold, aneuploid embryos were unable to attach to the endometrial cells, while euploid embryos attached.
What is known already: Although embryo selection for transfer is usually based on morphology, 70% of embryos with high morphological quality have chromosomal abnormalities. The results of implantation and pregnancy rate assessments following Preimplantation Genetic Screening (PGS) are controversial. There is still no *in vitro* study to compare the implantation of human euploid and aneuploid embryos.
Study design, size, duration: After informed consent, 10 endometrial biopsies were taken from fertile women. For scaffolding, the stromal cells were resided within the matrix, after 24 hours, the epithelial cells were seeded on the scaffold. Cell culture continued for 5 days to reach the appropriate confluence. The embryos were also examined by performing PGS following CGH Array. 10 euploid and 10 aneuploid blastocysts were selected and co-cultured for 72 hours with the 3D structure of human endometrial cells.
Participants/materials, setting, methods: Endometrial cells were isolated and expanded in 2D cultures to achieve enough cells. The fibrin-agarose scaffold was made and stromal and epithelial cells were cultured into and on the scaffold, respectively. Then, cell proliferation was assessed by MTT assay. The simulated endometrial construct was confirmed by H&E and IHC. Partial hatching of blastocysts was performed using a laser system. The blastocyst's attachment to the endometrial-like structure was examined under a phase-contrast microscope and SEM.
Main results and the role of chance: The MTT OD of scaffolds increased during 5 days of cell culture ($P < 0.05$). The histological evaluation of the co-culture systems was done under light microscopy by H&E staining. On the top of the 3D culture system, epithelial cells shaped a constricted cell monolayer. Stromal cells combined with the fibrin-agarose scaffold got lengthened and expanded, displaying that the 3D culture systems supplied a suitable environment for the growth of endometrial cells.

In the 3D culture, the origins and locations of epithelial and stromal cells were defined by cytokeratin and vimentin immunostaining, respectively. IHC for cytokeratin was only positive for epithelial cells in the surface epithelium. IHC for the vimentin was positive for the stromal cells located in the 3D matrix. These results showed that fibrin-agarose scaffold could simulate the human endometrial structure.

Using SEM and phase-contrast microscopy, it was found that only euploid embryos were able to attach to the endometrial construct while aneuploid embryos weren't.
Limitations, reasons for caution: Since the co-culture does not contain a unique cell type, and the MTT OD standard curve against cell number is specified for cell type, the number of growing cells in the co-culture cannot be calculated; therefore it is reported as OD.
Wider implications of the findings: Our findings determined that PGS allows us to transfer top-quality embryos with higher implantation potential. It improves implantation and pregnancy rate during ART cycles, especially in patients with recurrent implantation failure.
Trial registration number: not applicable

P-370 RPL-protease A as a potential biomarker for predicting recurrent pregnancy loss

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Study question: Could the reduction of RPL-protease A be involved in the dysfunctional trophoblast for resulting in recurrent pregnancy loss (RPL).
Summary answer: Low expression of RPL-protease A may result in RPL and low serum RPL-protease A level may be a potential biomarker for predicting RPL.
What is known already: The RPL-protease A is expressed and secreted by placenta. The RPL-protease A is involved in the pathogenesis of pre-eclampsia, and the serum RPL-protease A level is higher in the patients with pre-eclampsia than that of normal groups. In our previous study, we identified that the RPL-protease A mRNA level was lower in the villi of patients with RPL than that of normal groups.
Study design, size, duration: Using the CRISPR/Cas9 system, the RPL-protease A gene knockout BeWo cell (BeWo KO) line was established, and the wild type (BeWo WT) and BeWo KO cells were applied to investigate the roles of RPL-protease A in trophoblasts. The human serum RPL-protease A levels were investigated by Western blot analysis and ELISA kit.
Participants/materials, setting, methods: The cell-cell fusion, cell counting analysis, invasion and scratch wound assays, cell cycle analysis, and immunocytochemical analysis were used to investigate cellular functions of RPL-protease A in trophoblast. The sera were obtained from 32 normal pregnant women and 60 women with RPL. The Western blot analysis and ELISA were used for detection of serum RPL-protease A levels.

Main results and the role of chance: The β-hCG was detected in fused BeWo WT cells, while the BeWo KO cells cannot fuse and did not express the -hCG. The ability of invasion was decreased, but the capacity of migration and proliferation was higher in BeWo KO cells than BeWo WT cells. Cell fusion related factor (-hCG), and cell invasion related factors (MMP-2 and MMP-9) were highly expressed in BeWo WT cells, and cell related factor (FAK), and cell proliferation related factors (ERK, p38, JNK, MKK3, MKK6, Raf, and Ras) were highly expressed in BeWo KO cells. The Western blot analysis and ELISA indicate that the serum RPL-protease A level was decreased in patients with RPL compared to that of normal groups.
Limitations, reasons for caution: The results of this study have the limitation of RPL-protease A functions in vitro.
Wider implications of the findings: The cellular functions of RPL-protease A in trophoblasts were investigated to explain the pathogenesis of RPL, and low serum RPL-protease A level can be used for a potential biomarker predicting RPL.
Trial registration number: not applicable

P-371 Clinical value assessment between endometrial receptivity array and immune profiling in patients with implantation failure

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Study question: To evaluate whether the pregnancy outcomes could be improved in implantation failure patients by endometrial receptivity array, endometrial immune profiling, or a combination of both.
Summary answer: There was no statistical difference between different endometrial receptivity evaluation and treatment in improving the clinical pregnancy rate.
What is known already: Both endometrial receptivity array and endometrial immune profiling were promised to improve the endometrial receptivity and subsequent clinical pregnancy. However, less is known about the efficiency between each other and whether the combination could further enhance their clinical value.
Study design, size, duration: Between November 2019 and September 2020, 143 women with a history of at least two or more consecutive implantation failure in IVF/ICSI treatment in Chengdu Xinan Gynecology Hospital were included. They were divided into three groups: 'ERA + Immune Profiling' (n = 70), 'Immune Profiling' (n = 41), and 'ERA' (n = 32).
Participants/materials, setting, methods: Inclusion criteria were age ≤ 38, with normal uterus and uterine cavity. All patients were suggested to evaluate endometrial receptivity by ERA test (Igenomix, Valencia, Spain) and endometrial immune profiling based on immunohistochemistry simultaneously, who would be free to choose each or both evaluation approaches. Personal Embryo Transfer and/or personal medical care were adopted according to evaluation results. Clinical pregnancy was confirmed by gestational sacs observed under ultrasonography.
Main results and the role of chance: The overall prevalence of displaced window of implantation (WOI) is 84.3%, and nearly 74.8% (83/111) patients were diagnosed as endometrial immune dysregulation. Clinical Pregnancy rate and embryonic implantation rate decreased in the 'Immune Test' groups, but without a statistical difference (P = 0.311, and 0.158, respectively). Multivariable logistic regression analysis showed that different endometrial receptivity evaluation and treatment was not associated the clinical pregnancy rate, suggesting the performance of different endometrial receptivity evaluation and treatment is similar in improving the clinical pregnancy rate. Neither the immune profiling (CD56, P = 0.591; FOXP3, P = 0.195; CD68, P = 0.820; CD163, P = 0.926; CD1a, P = 0.561; CD57, P = 0.221; CD8, P = 0.427; CD138 CE, P = 0.372) nor histologic endometrial dating defined by Noyes criteria (P = 0.374) were associated with ERA phases.
Limitations, reasons for caution: Although the selection of evaluation approaches was based on patients' willingness, the variances of baseline characteristics and immune profiling existed in different groups. The immunological treatment efficacy based on immune profiling was not evaluated before embryo transfer.
Wider implications of the findings: To our knowledge, this is the first study comparing the pregnancy outcomes after two typical endometrial receptivity evaluation approaches. The findings highlight the unsubstitutability for each assessment, indicating that both asynchronous and pathological WOI contribute to implantation failure.
Trial registration number: X2019004

P-372 Maternal circadian disruption is associated with impaired rhythmic expression of molecular clock genes and IUGR during placenta development in mice.

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Study question: Are molecular clock genes (MCGs) expressed rhythmically in mouse placenta, and whether maternal circadian rhythm disruption (MCRD) is associated with intrauterine growth retardation (IUGR) through disturbing rhythmic expression of MCGs?
Summary answer: Maternal circadian disruption causes impaired rhythmic expression of MCGs (*Bmal1, Clock, Npas2, Per1, Per2, Per3, Cry1, and Cry2)* and IUGR during placenta development in mice.
What is known already: The world economy is based on a 24/7 society and shift work or jet travel across time zones disrupts circadian rhythm in pregnant women. Evidence indicates that gestational chrono-disruption results in IUGR. Mature mouse and human placenta express MCGs. There is no information in the literature on whether the MCG expression in the placenta is rhythmic or not and whether the rhythmic expression of MCGs is impaired due to MCRD during pregnancy. Also, it is not known whether the association with MCRD and IUGR is related to MCGs.
Study design, size, duration: Young adult female BALB/c mice were paired with males until vaginal plug formation was verified. Females were randomly assigned to two groups: control and phase-advance. Controls remained on a constant 12-hr light:12-hr dark cycle, whereas phase-advanced mice were subjected to 6-hr advances in the LD cycle every 5 days. Placentae (n = 1329) and fetuses were obtained from 144 mice at Zeitgeber time (ZT)0, ZT6, ZT12, and ZT18 days 12, 14, and 16 of pregnancy.
Participants/materials, setting, methods: The following analysis was performed: (i) open field test was used for locomotor activity evaluations to confirm MCRD, (ii) placenta/fetus weight ratio for evaluation of IUGR development, (iii) morphometric evaluation of placental compartments utilizing H&E staining (iv) gene expression analysis of MCGs utilizing qRT-PCR. One-way and Two-way ANOVA test followed by Holm-Sidak posthoc test was used for multiple comparisons. Values are expressed as mean ± standard error, and values below $p<0.05$ were considered statistically significant.
Main results and the role of chance: Expression of MCGs (Bmal1, Clock, Npas2, Per1, Per2, Per3, Cry1, and Cry2) was rhythmic in the early and mature placenta development stages (days 12, 14, 16). Locomotor activity tests reveal that the total distance covered on the 16th day of pregnancy significantly decreased compared to the control group ($p=0.000158$). The ratio of the time spent in the outer/inner quadrant, an anxiety indicator, significantly increased in the MCRD group on the 14th ($p=0.0351$) and 16th days of pregnancy ($p=0.000329$). While the number of fetuses was similar in both groups for all gestational days ($p=0.896$), in the MCRD group, the fetus/placenta weight ratio decreased significantly on the 12th and 16th days of pregnancy ($p<0.001$). Thus, IUGR developed due to MCRD. Histomorphometry analysis of the placental compartments revealed a significant reduction in the spongiotrophoblast layer's size on all days of pregnancy and the labyrinth layer on day 16 ($p<0.05$). Finally, the rhythmic expression MCGs were impaired in placentas obtained from MCRD groups on days 12th, 14th, 6th of pregnancy ($p<0.001$). In conclusion, we found a robust relationship with the disturbed MCGs expression and occurrence of IUGR during a chrono-disrupted gestation.

Limitations, reasons for caution: Since this study was conducted in mice, care should be taken when translating the results to humans.
Wider implications of the findings: Our results in mice are important for initiating basic science knowledge regarding the outcomes of maternal chrono-disruption. Moreover, research in the placenta of gestational chrono-disrupted mothers, such as shift-workers, are urgently needed to translate our findings into the clinic.
Trial registration number: TUBITAK-119S121 and Akdeniz University Research Projects Unit TYL-2018-3960

P-373 Initial β-hcg levels and the increase rates after 2 days are effective in predicting pregnancy outcomes in single blastocyst transfer in frozen-thawed or fresh cycles

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Study question: What is the role of the initial β-hCG value and the β-hCG rate of increase after 2 days in the predictability of the pregnancy outcomes?
Summary answer: The initial β-hCG values and β-hCG increase rates after 2 days are effective in predicting early pregnancy loss (EPL) and live birth rates (LBR).
What is known already: β-hCG is a glycoprotein-structured hormone secreted by the cyto syncytiotrophoblasts of the blastocyst and detected in the blood at the earliest 6-8 days after fertilization. The β-hCG level increases approximately by doubling in 48 hours in normal pregnancies. There are few studies conducted about the initial β-hCG values and the increase rates after 2 days on the pregnancy outcome of ART cycles and these studies indicated different threshold values and its level still remains unclear.
Study design, size, duration: This is a retrospective cohort study and was conducted at IVF and Reproductive Genetics Centre, Memorial Sisli Hospital, Istanbul, Turkey between January 2016 and December 2019. A total of 4605 including 3834 FET and 771 Fresh cycles resulted in a positive pregnancy test after a single blastocyst transfer was examined.
Participants/materials, setting, methods: The initial β-hCG test was applied 9 days after ET, when is the 14th day after ovulation. The latter β-hCG test was applied 2 days later. The cases with missing initial β-hCG tests or second β-hCG tests and missing follow-up during pregnancy were excluded. The mean β-hCG values and the increase rates after 2 days of the cases who had biochemical pregnancy loss (BPL), EPL and achieved live birth were reviewed.
Main results and the role of chance: The mean initial serum β-hCG level on 9 days after ET in the live birth group was 185.51 ±97.38 IU/L in FET cycles, which was significantly higher than the groups of BPL (62.75 ±50.70 IU/L) and EPL (133.93 ±95.10 IU/L). However, in fresh cycles, these levels in the live birth group was 167.70 ±114.05 IU/L which was significantly higher than the groups of BPL (50.13 ±27.49 IU/L) and EPL (106.55 ±71.80 IU/L).

The mean β-hCG levels in FET cycles were significantly found higher than in fresh cycles (P< 0.005) regardless of pregnancy outcomes.

The β-hCG threshold value predicting live birth for fresh cycle was found 108 IU/L (sensitivity 71.5 %, specificity 70.8 %, PPV 87. % and NPV 46.3%), while this value was found 101 IU/L (sensitivity 81.4 %, specificity 55 %, PPV 81.6 % and NPV 53.8 %) for FET cycles.

The β-hCG increase rate of threshold value predicting LBR for fresh cycle was 1.92 (sensitivity 90.6 %, specificity 36.3 %, PPV 80.1% and NPV 57 %), while this rate was found 2.01 (sensitivity 90.1 %, specificity 38%, PPV 78.4% and NPV 61.1 %) for FET cycles.

The β-hCG increase rate was not different between fresh and FET cycles.
Limitations, reasons for caution: Retrospective study
Wider implications of the findings: The initial β-hCG values and the increases in β-hCG values after 2 days can be used as effective parameters in the diagnosis of pregnancy outcomes. Early prediction of pregnancy outcomes may help to the clinician to manage and follow-up high risk pregnancies.
Trial registration number: Not Applicable

P-374 Investigating causality of risk factors for miscarriage – a Mendelian randomization analysis

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Study question: Do modifiable risk factors such as smoking, alcohol or coffee consumption, and adiposity causally increase the risk of sporadic or recurrent miscarriage?
Summary answer: We found evidence for a causal relationship between smoking initiation and sporadic miscarriage, but not for any other risk factor tested.
What is known already: Miscarriage is estimated to end between 10-25% of clinically confirmed pregnancies, and many observational studies have suggested numerous lifestyle factors, such as coffee and alcohol consumption, smoking and increased adiposity, may increase miscarriage risk. However, results are not always consistent across studies, and definitive causal relationships between various risk factors and miscarriage have not yet been demonstrated. Mendelian randomization utilizes genetic variants significantly associated with heritable risk factors (*i.e.* at *P*-values <5x10-8 in large genome-wide association studies) as instrumental variables to investigate causality of risk factors in population health outcomes.
Study design, size, duration: We conducted two-sample Mendelian randomization analyses to investigate causality of smoking (initiation and quantity), alcohol and coffee consumption (quantity), and adiposity (body mass index and waist-hip ratio) in sporadic and recurrent miscarriage. Data included in this study were taken from previously published summary genetic association statistics (betas, standard errors and *P*-values) from large-scale genome-wide association studies (GWAS) for each risk factor, and from our recently published GWAS of sporadic and recurrent miscarriage.
Participants/materials, setting, methods: Instrumental variables were constructed using 5-306 genetic variants significantly associated with the listed risk factors in published GWAS (minimum N=178,000 individuals). Two instrumental variables were constructed per risk factor using data from different GWAS. Associations of the instrumental variables with miscarriage were investigated using summary association data from women of European ancestry included in our miscarriage GWAS, including 49,996 sporadic miscarriage cases and 174,109 female controls, and 750 recurrent miscarriage cases and 150,215 female controls.
Main results and the role of chance: We found a significant association between sporadic miscarriage and the instrumental variables for two smoking measures: smoking initiation (inverse variance weighted Odds Ratio = 1.17, 95% confidence intervals = 1.10-1.24, *P* = 2.7 x 10-07) and lifetime smoking (inverse variance weighted Odds Ratio = 1.22, 95% confidence intervals 1.11-1.35, *P*=4.2x10-5). No other risk factors (smoking quantity, coffee or alcohol consumption, or BMI or waist-hip ratio) were associated with either sporadic or recurrent miscarriage. *A priori* power calculations considering the amount of phenotypic variance in each risk factor explained by the associated SNPs suggested our analysis to have at least 75% power to detect an association with Odds Ratio of 1.2 with sporadic miscarriage for analyses of body mass index, waist hip ratio and smoking initiation, quantity and the lifetime smoking measure, but that the alcohol and coffee consumption analyses were underpowered (4.9% and 48%, respectively). All analyses were underpowered for recurrent miscarriage given the small case sample size (N=750).
Limitations, reasons for caution: While data utilised here come from large-scale GWAS including 1000s of individuals, genetic variants significantly associated with each risk factor currently explain small percentages (0.02-6%) of the variance in each trait. Larger GWAS for specific risk factors, and for

sporadic and recurrent miscarriage, are required to clarify some published associations.
Wider implications of the findings: We find no evidence of a causal link between adiposity and miscarriage, indicating that observational findings of increased miscarriage risk with increasing body mass index require further explanation. Significant associations between measures of ever-smoking and sporadic miscarriage highlights that no amount of smoking is safe in regards to miscarriage risk.
Trial registration number: Not applicable

P-375 Intralipid supplementation in women with unexplained recurrent implantation failure and elevated uterine natural killer cell levels - A randomized placebo controlled trial

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Study question: Does intralipid supplementation in women with unexplained recurrent implantation failure (RIF) with elevated uterine natural killer cell (uNK) levels improve pregnancy outcomes during IVF?
Summary answer: Intralipid supplementation appears to improve clinical pregnancy rate in women with unexplained RIF with elevated uNK cell levels.
What is known already: The increased numbers of uNK cells in peri-implantation endometrium have been reported in women with recurrent miscarriage (RM) and RIF after IVF. However, reports are contradictory when it comes to correlation of increased numbers of uNK cells with pregnancy outcome. Current opinion suggests there is a potential for intralipid therapy in improving reproductive outcome, although data on live birth rate is very limited. No studies have assessed the effect of intralipid on IVF outcomes in RIF women based on elevated uNK cells. Identified studies have all used pNK cell testing as preferred diagnostic tool for analysis of NK cell levels.
Study design, size, duration: A randomized placebo controlled trial was conducted at Division of Reproductive Medicine at tertiary care institute. Thirty women with RIF and fifty fertile controls with age <35 years having regular menstrual cycles and no hormonal treatment in last 3 months were enrolled in the study from January 2019 to December 2020 for uNK cell testing. Randomization was done using random numbers and sealed envelopes. Only the subjects were masked and allocation concealment was done.
Participants/materials, setting, methods: Subjects included RIF 20-35 years, normal ovarian reserve, unexplained and tubal factors, normal karyotype and normal uterine cavity. Cut off for uNK cells was derived from fertile controls by immunohistochemical staining of CD56+ cells from midluteal endometrial biopsy sample. Subjects with elevated uNK cell levels were randomized during IVF to group A (Intralipid) or group B (saline). The infusion was repeated within one week of positive pregnancy test and then every 2 weeks.
Main results and the role of chance: The mean age and BMI were comparable between fertile control and study group(29.45±3.3 vs 31.17±3.3 years, 22.97±1.89 vs 23.21±2.2 kg/m2 ;p>0.05). The median uNK cell levels was 7%(used as cut off) in fertile controls and 13.5% in RIF. 18 women (60%, 18/30) with RIF who had elevated uNK cell level (>7%) were randomized. Four women were lost to follow up. The median age, BMI, number of previous failed cycles and duration of infertility were comparable between Group A(n=7) and Group B(n=7){30(IQR:27-31) vs 33(IQR:30-34)years, 22.7(IQR:21.08-24.4) vs 22.6(IQR:21.37-24.2)kg/m2, 2(IQR:2-3) vs 2(IQR:2-3), 8(IQR:7-8) vs 8(IQR:7-10)years}. The median FSH, AMH and AFC were 5.86(IQR:5.13-7.67)mIU/l, 2.4(IQR:2.16-6.12)ng/ml, 10(IQR:8-12) in Group A which were comparable with Group B {6.2(IQR:4.78-6.5)mIU/l, 4.8(IQR:2.67-6.25)ng/ml, 12(IQR:12-16) }. All patients underwent antagonist protocol. The clinical pregnancy rate was 57.14%(4/7) in group A which was significantly higher as compared to 28.6%(2/7) in group B(p<0.05). None of the patients reported any side effects due to intralipid.
Limitations, reasons for caution: The limitation of present study is its small sample size. However, the study is currently recruiting more RIF patients, and these are the interim results of the same. More RCTs with larger sample size are required to assess the efficacy of intralipid in this specific subset of population.
Wider implications of the findings: The present study suggests the beneficial effect of intralipid in women with unexplained RIF with elevated uNK cell levels in increasing the chemical and clinical pregnancy rate. However, ongoing pregnancy rate and live birth rate should be investigated further in this subset of population.
Trial registration number: CTRI/2019/01/017213

P-376 S100P in syncytiotrophoblast regulates lipid metabolism during early pregnancy

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Study question: Does S100P involve in the regulation of lipid metabolism in trophoblast syncytialization during the early stage of pregnancy?
Summary answer: S100P suppressed lipid droplets overloading in trophoblast syncytialization during the early stage of pregnancy.
What is known already: S100P is a 95-amino acid protein belongs to the large family of S100 calcium-binding proteins and is exclusively expressed in syncytiotrophoblast layer of placenta, enhancing trophoblast cells proliferation, motility and invasion. Recent studies indicated S100P was aberrantly expressed in various malignant tumors, promoting their proliferation, survival, the formation and invasion of vessels, and has a close relationship with their grade malignancy, hormone dependency and the drug-resistance. Syncytialization is trophoblast cells differentiated from cytotrophoblasts fused into multinucleated cell clusters with cell boundaries deficiency.
Study design, size, duration: Tissues were collected from elective first trimester pregnancy terminations of healthy people and patients with recurrent spontaneous abortion. Human trophoblast stem cells (hTSCs) were isolated from Week 6 to Week 8 of fresh placental tissues (n=8) and use forskolin to induce syncytialization.
Participants/materials, setting, methods: Tissues were collected from elective first trimester surgical pregnancy terminations to determine localization, abundance and function of S100P. The level of S100P protein and gene was measured by Real-time quantitative PCR (RT-qPCR), western blot, ELISA and immunofluorescence in hTSC induced ST(2D)-TSCT. The lipid droplets were assessed by Oil red O stain, BODIPY and electron microscope. Serum S100P concentration in normal and recurrent miscarriage patients were measured by ELISA.
Main results and the role of chance: S100P was exclusively localized in syncytiotrophoblast layer of the placental villous from gestational weeks 6–8 (n=4); S100p serum concentration was increased markedly as the gestation progressed (n=40, p<0.05); the protein abundance of S100P was increased during syncytialization of hTSCs; Lipid droplets increased during syncytialization and knockdown of S100P leads to trophoblast cell death with the overload of lipid droplet; The expression of S100P was down regulated and the amount of lipid droplets apparently accumulated in villus of recurrent miscarriage patients (n=4).
Limitations, reasons for caution: The role of S100P on placenta lacks the evidence from animal models.
Wider implications of the findings: S100P is an important factor facilitating in trophoblast syncytialization in the first trimester of pregnancy and could be a biomarker of the early pregnancy sustenance.
Trial registration number: not applicable

P-377 Association between antinuclear antibodies and pregnancy prognosis in recurrent pregnancy loss patients

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Study question: Can antinuclear antibody (ANA) affect the subsequent live birth rate in patients with recurrent pregnancy loss (RPL) who have no antiphospholipid antibodies (aPLs)?
Summary answer: ANA did not affect the pregnancy prognosis of RPL women.

What is known already: The prevalence of ANA is well-known to be higher in RPL patients. Our previous study found no difference in the live birth rates of ANA-positive and -negative patients who had no aPLs. Higher miscarriage rates were also reported in ANA-positive patients compared to ANA-negative patients with RPL. The RPL guidelines of the ESHRE state that "ANA testing can be considered for explanatory purposes." However, there have been a limited number of studies on this issue and sample sizes have been small, and the impact of ANA on the pregnancy prognosis is unclear.
Study design, size, duration: An observational cohort study was conducted at Nagoya City University Hospital between 2006 and 2019. The study included 1,108 patients with a history of 2 or more pregnancy losses.
Participants/materials, setting, methods: 4D-Ultrasound, hysterosalpingography, chromosome analysis for both partners, aPLs and blood tests for ANA and diabetes mellitus were performed before a subsequent pregnancy. ANAs were measured by indirect immunofluorescence. The cutoff dilution used was 1:40. In addition, patients were classified according to the ANA pattern on immunofluorescence staining. Live birth rates were compared between ANA-positive and ANA-negative patients after excluding patients with antiphospholipid syndrome, an abnormal chromosome in either partner and a uterine anomaly.
Main results and the role of chance: The 994 patients were analyzed after excluding 40 with a uterine anomaly, 43 with a chromosome abnormality in either partner and 32 with APS. The rate of ANA-positive patients was 39.2 % (390/994) when the 1: 40 dilution result was positive. With a 1:160 dilution, the rate of ANA-positive patients was 3.62 % (36/994). The live birth rate was calculated for 798 patients, excluding 196 patients with unexplained RPL who had been treated with any medication.

With the use of the 1: 40 dilution, the subsequent live birth rates were 71.34 % (219/307) for the ANA-positive group and 70.67 % (347/491) for the ANA-negative group (OR, 95%CI; 0.968, 0.707-1.326). After excluding miscarriages with embryonic aneuploidy, chemical pregnancies and ectopic pregnancies, live birth rates were 92.41 % (219/237) for the ANA-positive group and 92.04 % (347/377) for the ANA-negative group (0.951, 0.517-1.747). Using the 1:160 dilution, the subsequent live birth rates were 84.62 % (22/26) for the ANA-positive group, and 70.47 % (544/772) for the ANA-negative group (0.434, 0.148-1.273).

Subgroup analyses were performed for each pattern on immunofluorescence staining, but there was no significant difference in the live birth rate between the two groups.
Limitations, reasons for caution: The effectiveness of immunotherapies could not be evaluated. However, the results of this study suggest that it is not necessary.
Wider implications of the findings: The measurement of ANA might not be necessary for the screening of patients with RPL who have no features of collagen disease.
Trial registration number: not applicable

P-378 Using a machine learning tool (72% accuracy with 64% PPV) to predict multiple live birth when transferring multiple embryos, based on embryo specific data

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Study question: Can Machine Learning predict multiple pregnancy based on data specific to the embryos and the patient?
Summary answer: Embryo data are useful in determining which embryos are likely to lead to multiple pregnancy. Patient age has low predictive value compared to embryo data.
What is known already: Our previous assessment of the HFEA data demonstrated that single embryo transfer (SET) in the UK occurred in a minority (45%) of fresh cycles, with a marginal increase in live birth rate (LBR) in some patient cohorts in favor of multiple embryo transfer (MET). Current policies on determining number of embryos for transfer tend to be generic and do not account for detailed embryology data. Generic policies may compromise LBR for some patients that would benefit from MET. Artificial Intelligence has the potential to assist in this decision process.
Study design, size, duration: Retrospective cohort analysis from 2013 to 2020 of 193 cycles with 386 embryos used in double ETs on day 5 at POMA fertility clinic with positive live birth outcome. ML model, xgboost, was trained to predict multiple live birth (N=54) versus single live birth (N=139). Detailed embryology data from day 1 to day 5 were used as input.
Participants/materials, setting, methods: Input of the machine learning model included patient age and 18 morphological parameters collected on days 1, 2, 3 and 5 (symmetry, number of cells, blastocyst status, fragmentation, ICM and troph grades) from the two transferred embryos. An xgboost algorithm was trained on 80% of the data (n= 154) and tested on 20% of blind data (n=39).
Main results and the role of chance: Xgboost machine learning algorithm predicted multiple live birth on the blind dataset with an accuracy of 72%, with an AUC of 0.60, showing better results than random. PPV (true prediction of multiple births) was 64% and NPV (true prediction of single birth) was 75%.

The following parameters ranked high in the predictive power of the machine learning (in order of predictive power): blastocyst status on day 5 of both embryos, symmetry on day 3, number of cells on day 2, scores on day 2 and 3. Limitations, reasons for caution: The dataset was derived from a single clinic with manual annotations and may not be transferable to other clinics. The risk of bias is important as the model was trained only àon embryos that were transferred and led to at least one birth
Wider implications of the findings: A tool to help identify which patients are at increased risk of MP with MET would be clinically useful to help patients and clinical team make the best personalised decision for a specific embryo, finding the balance between maximising success rate whilst minimising multiple pregnancy rate and its associated risks.
Trial registration number: not applicable

P-379 Human platelet lysate improves trophoblast spheroid attachment to primary endometrial epithelial cells from patients with recurrent implantation failure

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Study question: Could non-autologous platelet lysate (PL) increase attachment of HTR-8 spheroids *in vitro* to primary endometrial epithelial cells (EECs) from patients with recurrent implantation failure (RIF)?
Summary answer: Increased quantity of HTR-8 spheroids attached to primary EECs, isolated from patients with RIF, suggests *in vitro* treatment with non-autologous PL could improve endometrial receptivity.
What is known already: Inadequate endometrial receptivity and thickness are major causes for RIF. Recent studies suggest that platelet-rich plasma (PRP) may improve pregnancy outcomes for RIF and/or thin endometrium (TE) patients. Our previous results show that a commercially sourced and non-autologous human PRP/PL (HPL) promotes EC proliferation *in vitro*, suggesting that HPL may help to standardize future clinical treatments. In addition to EC proliferation, HPL treatment may improve embryo attachment to primary EECs isolated from patients with a history of RIF. *In vitro* attachment assays with trophoblast spheroids (embryo model) could help elucidate the effect of HPL on endometrial receptivity in RIF patients.
Study design, size, duration: Endometrial tissue was collected from nine RIF patients at the CReATe Fertility Centre, Toronto, Canada (Veritas REB#16580): five with (RIF+TE) and four without a TE (RIF only). Primary EECs were enzymatically isolated and treated with serum-free culture media (SFM) or 1% HPL in SFM for 48 hours before performing the attachment assay. Trophoblast cells (HTR-8/SVneo) were grown in suspension on a rocker to form 70-100 uM spheroids over 24 hours before use in the assay.
Participants/materials, setting, methods: Spheroids were fluorescently labelled with calcein-AM for 30 minutes and size-selected to capture spheroids similar in size to a human blastocyst. Spheroids were seeded on top of EEC monolayers and calcein fluorescence was immediately measured by a spectrophotometer. Following the 1-hour incubation, unattached spheroids were aspirated, and fluorescence was measured again. Spheroids were also individually quantified by fluorescent microscopy and ImageJ™ software. The percentage of spheroid attachment was calculated for calcein fluorescence and ImageJ™ quantification.

Main results and the role of chance: The HTR-8/SVneo cell line, derived from human first-trimester extravillous trophoblast cells (EVT), has been shown to be a suitable cell line to assess adhesion and invasion *in vitro*. Trophoblast spheroids generated from this cell line visually resembled a blastocyst and maintained expression of the EVT and implantation biomarkers: GATA3, ITGA5, and LIF. Primary EECs, treated for 48 hours with SFM supplemented with 1% commercially sourced and non-autologous HPL, overall exhibited increased attachment to HTR-8 spheroids. The percentage of spheroid attachment, as measured by fluorescence alone, significantly increase from 47.98% to 64.27% ($P<0.01$) of seeded spheroids in RIF+TE EEC cultures, and from 48.12% to 85.77% ($P<0.001$) of seeded spheroids in RIF only EEC cultures. Quantification by fluorescent microscopy and ImageJ™ software for individual calcein-stained spheroids, revealed a significant increase in spheroid attachment, from 57.52% to 86.5% ($P<0.01$) in RIF+TE EEC cultures, and from 42.58% to 68.90% ($P<0.01$) in RIF only EEC cultures.

Limitations, reasons for caution: Although there was a positive correlation between calcein fluorescence and spheroid quantity, quantification by fluorescence alone may be unreliable due to the variable numbers of cells in each spheroid. Our data suggest a more precise increase in attachment is detected when quantified by fluorescent microscopy and ImageJ™ software.

Wider implications of the findings: We report a method for functional assessment of endometrial receptivity *in vitro*. HPL appears to promote implantation in RIF patients in a model of embryo attachment. We predict that the observed increase in attachment is due to increased endometrial receptivity gene expression, which will be our next investigative avenue.

Trial registration number: N/A

P-380 Differential concentrations of maternal and fetal hemopexin and α1-microglobulin in preeclampsia from IVF pregnancies depending on the presence of corpus luteum at embryo transfer

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Study question: Does the presence of corpus luteum (CL) in *in vitro* fertilization (IVF) treatments affect maternal and fetal concentrations of hemopexin and α1-microglobulin in preeclampsia?

Summary answer: Decreased hemopexin and increased 1-microglobulin levels in maternal and fetal blood in IVF pregnancies with absence of CL particularly in pregnancies complicated by preeclampsia.

What is known already:

- Pregnancies after frozen embryo transfer (FET) in programmed cycles have higher rates of hypertensive disorders of pregnancy, suggesting a link between the absence of CL in programmed cycles and adverse maternal outcomes.
- Cardiovascular function is impaired early in pregnancy in women conceiving by IVF treatments in the absence of CL.
- Plasma relaxin-2, a potent vasodilator and stimulus of decidualization, has been reported to be undetectable in a non-CL cohort, but markedly elevated in a multiple-CL cohort through pregnancy.
- Hemopexin and α1-microglobulin act as scavengers that eliminate free heme-groups responsible for hemoglobin-induced oxidative stress known to contribute to preeclampsia development.

Study design, size, duration: A case-control study of 160 singleton pregnancies recruited from 2016 to 2020, including 54 spontaneous pregnancies from fertile couples, 50 conceived by IVF following fresh embryo transfer (ET) and FET in natural cycle (presence of CL) and 56 IVF after fresh oocyte-donation or FET in programmed cycles (absence of CL). Pregnancies were subclassified according to the presence of preeclampsia in uncomplicated, preeclampsia and severe preeclampsia cases.

Participants/materials, setting, methods: IVF pregnancies were recruited from a single Assisted Reproduction Center, ensuring homogeneity in IVF stimulation protocols, endometrial preparation, laboratory procedures and embryo culture conditions. Spontaneous pregnancies from fertile couples were randomly selected from our general population and matched to IVF by gestational age at birth. Hemopexin and α1-microglobulin concentrations were measured by ELISA in maternal and cord plasma collected at delivery. All comparisons were adjusted for age, ethnicity, prematurity, birthweight centile, oocyte-donation and FET cycles.

Main results and the role of chance: Parental ethnicity, body mass index, exposure to aspirin and corticoids during pregnancy, mean gestational age at birth and birthweight were similar in all study groups. While maternal hemopexin levels were lower in treatments without CL, the IVF group with one or several CL showed significantly increased hemopexin concentrations, both in uncomplicated and preeclampsia cases (uncomplicated: spontaneous conceptions median 1520 ug/ml [interquartile range 1054-1746], IVF with CL 1554 [1315-1778], IVF without CL 1401 [1130-1750]; Preeclampsia: spontaneous conceptions 1362 [1121-1667], IVF with CL 1372 [403-2558], IVF without CL 1215 [971-1498]). Maternal α1-microglobulin was significantly higher in the absence of CL in severe preeclamptic cases as compared to spontaneous pregnancies and IVF with CL (spontaneous conceptions median 23 ug/ml [interquantile range 20-24], IVF with CL 24 [24-26], IVF without CL 26 [25-28]).

The cord blood profiles were identical to the maternal for both biomarkers.

Overall, and in line with previous studies, preeclamptic pregnancies independently of the mode of conception, showed decreased concentrations of hemopexin and increased concentrations of α1-microglobulin both in maternal and fetal plasma, with more pronounced changes in severe preeclampsia cases.

Limitations, reasons for caution: Infertility factors contribution to the outcome cannot be unraveled from the assisted reproductive technologies procedure itself as we have only included spontaneous pregnancies from fertile couples.

Adjustments for oocyte-donation and FET modalities were performed due to the higher proportion of these features in the ET in programmed cycles group.

Wider implications of the findings: These findings acknowledge physiological differences between pregnancies following ET in stimulated and natural versus programmed cycles, supporting the hypothesis that the CL activity could influence perinatal results.

This approach to perinatal outcomes in IVF patients could lead to changes in ET protocols in order to develop a CL if possible.

Trial registration number: not applicable

P-381 Deciphering the genetic cause of recurrent and sporadic pregnancy loss

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Study question: To investigate the prevalence and effect of (mosaic) *de novo* genomic aberrations in recurrent pregnancy loss (RPL) and sporadic abortion (SA).
Summary answer: Prevalence of maternal uniparental disomies (UPDs) was high in both cohorts. While chromosomal UPDs were found in both cohorts, genome wide UPDs were RPL specific.
What is known already: Spontaneous abortion occurs in 10-15% of clinically recognized pregnancies and recurrent pregnancy loss in 1-3%. SA and RPL are associated with reduced quality of life. Multiple factors contribute to SA and RPL, such as uterine malformations and parental/fetal chromosomal abnormalities. However, in ~60% of SA and RPL the cause remains unknown. UPD is defined as the presence of two homologues chromosomes originating from a single parent. This phenomenon can lead to imprinting disorders that are characterised by clinical features affecting growth, development and metabolism in liveborn offspring. However, it could also be responsible for pregnancy loss.
Study design, size, duration: We recruited 32 families with pregnancy loss (n=16 RPL cohort, n=16 SA cohort) with no known genetic predispositions and normal karyotyping results in both parents and the fetus. Average maternal age was 28.68 years (SD=5.43), paternal age 30.3 years (SD=5.53), and the gestational age at pregnancy loss was 8.65 weeks (SD=2.47). The average number of miscarriages in the RPL group was 3.57 (SD=0.84). We profiled the genomic landscape of both cohorts using SNP typing.
Participants/materials, setting, methods: We isolated DNA from blood of both parents and the placental tissues from the miscarried products of conception. The placenta tissues were sampled from two distinct extraembryonic and embryonic germ layers, the extraembryonic mesoderm and the chorionic villi cytotrophoblast. Subsequently, we performed SNP-genotyping using Illumina's Global-Screening Array-24 v2.0 BeadChips and applied haplarithmisis to delineate allelic architecture of fetal tissues of both cohorts. This allowed us to detect large *de novo* copy-number and -neutral (>10kb) changes.
Main results and the role of chance: In this pilot study, we have analyzed 132 DNA samples (n=32 families), of which 16 families were in the RPL cohort and 16 in the SA cohort. Within the RPL cohort, we found: one family with mosaic genome wide hexaploidy both in the extraembryonic mesoderm and chorionic villi, one family with a non-mosaic genome wide hetero UPD of the chorionic villi tissue, one family with a mosaic UPD of chromosome 14 in both tissues and tetraploidy exclusively in the chorionic villi, one family with a mosaic UPD of chromosome 16 in both tissues, one family with a mosaic UPD of chromosome 6 in both tissues, and another family with a mosaic UPD of chromosome 5 in the extraembryonic mesoderm. Within the SA group, one family showed a UPD of chromosome 7 and another family showed a segmental UPD of chromosome 5 in both tissues. Strikingly, all the UPDs found in this study were maternal in origin.
Limitations, reasons for caution: The main limitation of this study is the resolution of detecting copy-neutral and copy-number variations, which is an inherent limiting factor of SNP-array technology. In addition, in the sample in which we observed non-mosaic genome wide UPD, maternal contamination is likely that can be investigated by other technologies.
Wider implications of the findings: Multiple genome wide UPDs are found in the RPL group but none in the SA group, indicating an association between genome wide mosaic UPD and RPL. These findings could lead to a better understanding of causative factors for SA and RPL and the need for a SNP-based non-invasive prenatal testing.
Trial registration number: not applicable

P-382 Association of extended culture to blastocyst and gestational trophoblastic disease risk following IVF/ICSI assisted reproduction cycles: An analysis of large UK National database

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Study question: Is there any association between stage of embryo at transfer based on extended in vitro culture and gestational trophoblastic disease risk during assisted reproduction?
Summary answer: No significant association between stages of embryo transfer from zygote stage to blastocyst stage was found after analysing 540376 cycles of IVF and ICSI.
What is known already: Gestational trophoblastic disease (GTD), commonly referred to as molar pregnancy, results from abnormal proliferation of the trophoblast with a reported incidence of ~1 in 700 in the UK. Despite technological advances such as ICSI, PGT and selection of normally fertilised (2PN) embryos, there are reported cases of GTD following assisted reproduction. Blastocyst transfer is associated with higher pregnancy and live birth rates but evidence is lacking whether extended embryo culture to blastocyst stage influences implantation of an abnormal embryo or abnormal trophoblastic proliferation leading to GTD.
Study design, size, duration: A retrospective study was carried out using Human Fertilisation and Embryology Authority (HFEA) anonymised register data from 1999 to 2016. HFEA holds the longest running register for fertility treatment data in the world and is the national database for fertility treatment data in UK. A total of 540376 fresh IVF or ICSI assisted reproduction cycles were analysed.
Participants/materials, setting, methods: There were 1033588 treatment cycles during the study period but only 540376 cycles met the inclusion criteria of fresh IVF or ICSI. Cycles with incomplete data, frozen embryo transfers, donor treatment or surrogacy were excluded. A subgroup analysis of those with primary subfertility was performed after excluding subjects with secondary infertility in order to exclude an effect of a previous molar pregnancy. Multivariate regression analysis was used to adjust for possible confounders.
Main results and the role of chance: 78 molar pregnancies were reported in the original sample giving a prevalence of 4/10000 live births (78/228461), much lower than the prevalence given with natural pregnancies. Prevalence of molar pregnancy amongst the study population after meeting exclusion criteria was 4 /10000 livebirths (53/156683). Incidence of molar pregnancy was not statistically different between treatment type (0.0001 vs 0.00009). Significantly higher incidence of GTD was seen in the 40 to 42 age category compared to 18-34 category(OR 1.86(95% CI 8.7-3.75)),in par with known higher GTD risk in women above 40 in the general population. Of interesting note, although the incidence of molar pregnancy was significantly lower in women undergoing assisted reproduction increased risk with advancing age is not totally eliminated with treatment. There was no significant association between the occurrence of molar pregnancy with the type and cause for infertility and number of embryos transferred. Crude (1.06 (95% CI 0.852-1.31)) and adjusted odds ratios (1.07 (95% CI (0.857-1.32)) did not show any association between day of embryo transfer and molar pregnancy even after adjusting for age and excluding secondary infertility. Selection of blastocyst stage embryo after extended culture did not alter the likelihood of having a GTD compared to cleavage stage embryo.
Limitations, reasons for caution: The retrospective analysis of anonymised HFEA data limited adjustments for confounders such as smoking, previous history of GTD, ethnicity etc that predispose to GTD. Caution needs to be exercised for under-reporting of GTD to HFEA and lack of information on type of GTD identified.
Wider implications of the findings: Though GTD cannot be prevented by IVF/ICSI, the incidence is significantly low and extended culture is not associated with higher risk of abnormal trophoblastic proliferation or GTD occurrence with IVF/ ICSI treatment. These findings would aid informed implications counselling and reassurance of patients during assisted reproduction treatments.
Trial registration number: not applicable

P-383 Cytogenetic analysis of products of conception from miscarriages following natural conception and IVF pregnancy: a comparative study

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Study question: Do cytogenetic results from products of conception from miscarriages differ from patients conceiving by natural conception versus IVF?
Summary answer: Cytogenetic results were similar, with no statistical difference from miscarriages following natural conception and assisted conception.

What is known already: Cytogenetic sampling of products of conception (POC) following recurrent miscarriages (RM) are recommended to rule out parental chromosomal rearrangements. The RCOG recommends cytogenetic testing in cases of recurrent miscarriages (3 consecutive miscarriages). However some units routinely request cytogenetic analysis following a single miscarriage following an IVF pregnancy. There is no evidence to support the routine sampling of POCs following assisted conception. Study design, size, duration: Retrospective cohort study of 117 cytogenetic samples, followed up by the outcome of parental karyotyping if applicable. Patients were categorised based on mode of conception into natural conception (NC) with recurrent miscarriages (≥3) or one miscarriage following IVF. Data collected between 2018-2020. Primary Outcome measure: Presence and type of cytogenetic abnormality; individual parental targeted G-band karyotyping result. Participants/materials, setting, methods: A total of 117 cytogenetic results were reviewed, of which 35 were unsuitable for analysis due to contamination (Total *n*=79: NC = 60, IVF = 19). Main results and the role of chance: Cytogenetic analysis showed abnormal results in 59% of miscarriages following natural conception and 53% of miscarriages from IVF pregnancy (*p*=0.46).

Abnormal cytogenetic results were mainly sporadic. Trisomy 16 was the commonest abnormality in both groups. Others included Trisomy 15, 22, 21, 8, 13, 5, 9, 10, 14, 18, single X (Turner's), all occurring in the same frequency in both groups. As expected 35 out of 45 abnormal cytogenetic results occurred with a maternal age greater than 35 years.

One couple from the NC group were referred to a geneticist for a Trisomy 9 imbalance. All other parental karyotyping results were normal.

Limitations, reasons for caution: This study contains a small sample size, and would benefit from further data collection to account for a percentage of samples being inadequate for analysis. Wider implications of the findings: Cytogenetic results were similar from miscarriages following natural conception and assisted conception. IVF does not increase the risk of miscarriage from abnormal embryonic karyotype. Routine cytogenetic testing following one miscarriage in patients undergoing IVF is not cost effective.

Trial registration number: not applicable

P-384 First trimester pregnancy outcomes after confirmed SARS-CoV-2 infection in the community; a nationwide prospective longitudinal study of 10,000 pregnant women from the COVID-19 pandemic

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Study question: Are pregnant women in the community with confirmed diagnosis of SARS-CoV-2 infection, at increased risk of an early miscarriage?

Summary answer: Women diagnosed with COVID-19 in their first trimester were not at increased risk of an early miscarriage. What is known already: In the earliest stages of the pandemic, the Human Fertilisation and Embryology Authority and the European Society of Human Reproduction and Embryology, independently advised against starting assisted reproductive treatments. At the time of this recommendation, among other reasons, there were concerns about the complications of SARS-CoV-2 during pregnancy and the potential for vertical transmission.

We now having growing evidence that pregnant women are at an increased risk of severe illness along with higher rates of preterm births in those with severe acute respiratory syndrome. However, data on the impact of community infections of SARS-CoV-2 in early pregnancy has been sparse.

Study design, size, duration: This is an online survey study undertaken in the UK between May and November 2020. Pregnant women at any stage in their pregnancy were invited to participate in the study. Study participants were asked to complete online surveys at the end of each trimester. 10, 430 women were recruited to take part in the study. Participants/materials, setting, methods: We analysed pregnancy outcomes from women who were under 13 weeks gestation at the time of registration. We compared miscarriage rates among women with a confirmed diagnosis of SARS-CoV-2 infection to healthy controls. Those in the control group had not been diagnosed with or had symptoms of SARS-CoV-2 infection nor did they have any household contacts that were diagnosed with or had symptoms of SARS-CoV-2 infection.

Main results and the role of chance: 10, 430 pregnant women were recruited to participate in the study. 2934 were under 13 weeks gestation at the time of registration. The median age was 32.6 [IQR 29.8-35.6]. The median gestational age at registration was 8 weeks [IQR [6-10]. 246 women reported a miscarriage before 13 weeks of gestation. The overall miscarriage rate before 13 weeks of gestation was 8.4% (95% CI 7.3%-9.4%).

68 women reported a confirmed diagnosis of SARS-CoV-2 infection in their first trimester. The overall rate of confirmed SARS-CoV-2 infections in the first trimester was 2.3% (95% CI 1.8-2.9%). 3/68 (4.4%) were asymptomatic. Among those reporting symptoms, the commonest symptoms were fatigue (82%), headache (69%) and loss of smell/taste (69%). Only 38% of those with a confirmed diagnosis reported a fever. None of the 68 women with confirmed diagnosis of SARS-CoV-2 infection were hospitalised.

The rate of miscarriage before 13 weeks of gestation in women who were diagnosed with SARS-CoV-2 infections was not significantly higher compared to healthy controls (11.8% versus 9.3%, p = 0.35). A further 35 women had household contacts with confirmed SARS-CoV-2 infection although they themselves had not been diagnosed. No miscarriages were reported in this group.

Limitations, reasons for caution: None of the 68 patients diagnosed with SARS-CoV-2 were hospitalised. We do not know whether the rate of miscarriage among hospitalised women with SARS-CoV-2 infection is the same as those with community infections.

Wider implications of the findings: The overall rate of miscarriage during the pandemic was not higher than rates occurring outside of the pandemic. The rate of miscarriage among women diagnosed with SARS-CoV-2 infection was not significantly higher compared to healthy controls. This data can be used to counsel women planning a pregnancy during this pandemic

Trial registration number: not applicable

P-385 The relationship between systemic oestradiol and vaginal microbiota composition in miscarriage and normal pregnancy

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Study question: Is there an association between serum oestradiol, vaginal microbial composition and pregnancy outcome in the early first trimester?

Summary answer: In women with a vaginal microbiome deplete of *Lactobacillus species* at the time of Pregnancy of Uncertain Viability (IPUV), higher serum oestradiol associates with livebirth.

What is known already: During pregnancy, oestradiol mediates vaginal mucosal properties and increases glycogen deposition in epithelial cells which is thought to support colonisation of *Lactobacillus* species. Low levels of *Lactobacillus* associates with adverse outcomes such as miscarriage and preterm birth. The direct relationship between systemic oestradiol and the vaginal microbiome has never been studied in pregnancy. However studies have shown a positive correlation between serum oestrone, vaginal glycogen and *Lactobacillus* abundance in menopausal women.

Study design, size, duration: This was a prospective cohort study where one-hundred women were recruited in early pregnancy at the time of IPUV and donated paired blood and vaginal samples. 40 women had an eventual miscarriage, 58 had a livebirth and two pregnancies were terminated. All 100 women donated one paired serum and vaginal sample at this time point, and 22 women with *Lactobacillus* depletion at the time of IPUV donated further longitudinal vaginal samples.

Participants/materials, setting, methods: Participants were recruited from an Early Pregnancy Unit and underwent transvaginal ultrasound assessment of their pregnancy. Serum samples were analysed with an immunoassay on a

ROCHE COBAS E411 analyser for Oestradiol (pg/ml) and Progesterone (ng/ml). Bacterial DNA was extracted from paired vaginal swabs and sequenced using Illumina MiSeq sequencing of 16S rRNA gene amplicons.

Main results and the role of chance: *Lactobacillus* dominance of the vagina was associated with higher serum levels of E2 and progesterone compared to depletion (E2=398pg/ml vs 302pg/ml(p=0.02), P4=23.1ng/ml vs 17ng/ml(p=0.02)). E2 and P4 were positively correlated (r=0.6, p<0.05). At species level, *L. crispatus* dominance associated with significantly higher levels of E2 compared to high-diversity communities (468pg/ml vs 302pg/ml(p=0.03) but no such relationship was observed for P4. Both E2 and P4 levels were lower in women who eventually miscarried. However there was no significant difference in the vaginal bacterial composition at genera or species level at this early gestational age (P=0.08) regardless of per vaginal bleeding. However in women with *Lactobacillus* depleted microbiota, livebirth was associated with significantly higher E2 levels compared to women suffering miscarriage (212pg/ml in miscarriage vs 395pg/ml in livebirth, p=0.003) (OR=22.4 P=0.004). In 22 women who had Lactobacillus depletion at the time of IPUV (7 with an eventual outcome of miscarriage, and 15 with an eventual outcome of livebirth), longitudinal vaginal bacterial DNA sequencing was performed. In 7/15 women with livebirth, and higher E2 levels, the microbial composition changed to become more *Lactobacillus* dominant during pregnancy, whereas in those with miscarriage, only 1/7 changed to become *Lactobacillus* dominant.

Limitations, reasons for caution: In this study, serum oestradiol levels were compared to the local vaginal bacterial environment. The ideal would be to study local vaginal oestradiol, glycogen and the bacterial composition.

Wider implications of the findings: In contrast to previous studies in menopause where low oestrogen levels associate with the vaginal microbial composition, this study uses the high oestradiol environment of early pregnancy to study the mechanistic relationship between oestradiol and vaginal *Lactobacillus* abundance.

Trial registration number: NA

P-386 Uterine Natural Killer Cell function in Recurrent Miscarriage and Implantation Failure: A Systematic Review

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Study question: Does uterine natural killer cell functional activity differ in women with recurrent miscarriage (RM) or implantation failure (RIF) compared to fertile controls?

Summary answer: There is insufficient data to conclusively determine differences in uterine natural killer (NK) cell activity between women with RM/RIF and controls.

What is known already: Uterine NK cell (uNK) function is central to maintaining healthy pregnancy by promoting placentation and spiral artery vascular remodelling.

The range of uNK activity is diverse and includes cytokine secretion, such as IFN-γ, or angiogenic factors which promote vascular remodelling. Despite an abundance of studies investigating peripheral blood NK cell cytotoxicity, there is limited evidence of uNK cytotoxicity. uNK-trophoblast interactions are facilitated by uNK receptors such as CD94, LILRB1 and KIRs.

It is possible that dysfunction of these diverse uNK activities plays a more important role in early pregnancy failure than uNK levels.

Study design, size, duration: We conducted a systematic review of prospective case-control studies investigating uterine natural killer cell activity in patients with RM or RIF versus controls.

The aim was to determine whether there was a distinct variation in uNK activity between RM/RIF and controls. We stratified uNK activity into four broad categories: i) regulation and receptors; ii) cytotoxicity; iii) expression of cytokines; iv) effect on uterine vasculature.

Participants/materials, setting, methods: The electronic database search included MEDLINE, EMBASE, Web of Science and bibliographies from included articles from inception to December 2020 using a combination of MESH and keywords. Search, screen, and data extraction were performed by two reviewers independently. Quality assessment was conducted with ROBINS-I. Out of 4636 studies screened, 30 studies (1696 women) were analysed for uNK activity.

Main results and the role of chance: Different methods were used to measure uNK activity including immunohistochemistry, flow cytometry, ELISA, PCR and Western blot. Samples were obtained from endometrium during mid-luteal phase or decidua following surgery.

14 studies reported on uNK phenotypes associated with regulation and receptors. RM/RIF patients, compared to controls, demonstrated a reduced expression of KIR2DL4, KIR2DL3/L2/S2 and inhibitory receptors (NKG2A); whereas there was a higher expression of the activating receptor (NKp46) and CD161. One study reported correlation between FoxP3+ T-cells and CD56+ NK cells but another reported no similar correlation with CD57+/CD56+ ratio.

Two studies investigating dNK cytotoxicity, using chromium release or lactate dehydrogenase release assays, concluded higher dNK cytotoxicity in RM patients.

Eight studies reported on cytokine expression. Interestingly, two studies found lower expression of IFN-g, but four studies reported otherwise in RM patients. Two studies found a higher ratio of dNK producing IFN- /TNF- to those producing IL-4. The rest of the studies reported lower expression of IL-1RA, IL-10, TNF- , Lnc-49A and higher expression of granzyme B, perforin and PRF-1.

Finally, six studies reported on effect of uNK on vasculature. Among the findings were negative correlation between CD16+ uNK and endometrial IL-6 and VEGF, as well as higher angiogenin, VEGF and bFGF expression.

Limitations, reasons for caution: Functional activity investigated amongst studies varied significantly; this heterogeneity precluded meta-analysis of the data. Heterogeneity also prohibited definitive conclusions on uNK function and pregnancy outcome.

Studies presented data in a combination of qualitative and quantitative analysis and variation was seen in the criteria for inclusion of RM, RIF and controls.

Wider implications of the findings: uNK levels alone may be insufficient to guide management of early pregnancy failure. Clarification of underlying functional activity may guide therapeutic intervention and help develop new treatments.

This review highlights the need for a greater understanding of the role of uNK activity in healthy pregnancy and early pregnancy failure.

Trial registration number: N/A

P-387 Serum progesterone levels on the day of the endometrial receptivity analysis (ERA) biopsy do not correlate with the biopsy results

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Study question: Do the serum progesterone levels on the day of the endometrial receptivity analysis (ERA) biopsy correlate with the results of the ERA?

Summary answer: Serum progesterone levels on the day of the endometrial receptivity analysis biopsy do not correlate with the biopsy results.

What is known already: Endometrial receptivity is a time sensitive window characterised by maturation of the endometrium, during which the trophectodermal cells attach to the endometrial cells and invade the endometrial stromal vasculature. Progesterone is an essential element for receptivity and pregnancy. There is no consensus regarding the optimal progesterone levels in the luteal phase, for a successful pregnancy. Endometrial receptivity analysis is a diagnostic tool developed by profiling the transcriptome of over 238 genes that are expressed at different stages of the endometrial cycle. The results are reported as receptive, pre-receptive, early receptive, etc and are used to direct a personalised embryo transfer.

Study design, size, duration: We report a prospective study of 30 patients with a history of recurrent implantation failure (RIF). They underwent ERA testing in a medicated cycle, between early 2018 and late 2020.

Participants/materials, setting, methods: A large proportion of the patients we treat in our clinic (ARGC) have recurrent implantation failure. Thirty patients with RIF underwent ERA testing in a medicated cycle.They all followed the same protocol with down regulation, followed by estrogenic preparation for about 12-14 days, followed by progesterone for about 120 hours. An endometrial biopsy was taken at about 120 hours after progesterone exposure.

Main results and the role of chance: An ERA result was available on 28/30 patients. Eighteen were reported to be pre-receptive, seven receptive, 3 early receptive and 2 could not be analysed. The progesterone levels within 24 hours

of the biopsy for the pre-receptive group ranged from 21.2-472 nmol/l, for the receptive group ranged from 27.8-152 nmol/l and for the early receptive group ranged from 54.9-162 nmol/l. Though the number of cases is small, we found no co-relation between the serum progesterone levels with the ERA results. Eighteen women underwent an embryo transfer based on the ERA results (pET-personalised embryo transfer). Eleven were positive with four live births, one early ongoing pregnancy, three miscarriages, one ectopic pregnancy, two biochemical pregnancies and seven negative results. Seven women had euploid embryo transfers-three had live births, one is viable at 11 weeks, one had a missed miscarriage and two were negative. There are no studies correlating the serum progesterone levels and the ERA results. In practice, we plan embryo transfers for women in frozen cycles by monitoring the serum progesterone levels alongside the day of the cycle. Hence, we wanted to review if the combination of the progesterone levels along with biopsy results would allow us the improve the results further.

Limitations, reasons for caution: This is a small study. Larger datasets are required to draw meaningful conclusions.

Wider implications of the findings: If the above findings are confirmed by larger studies, we may not need to monitor serum progesterone levels during ERA biopsy cycles.

Trial registration number: NA

P-388 Endometrial extracellular vesicles from recurrent implantation failure patients inhibited embryonic growth and implantation via miR-6131/PAK2 pathway

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Study question: Could endometrial extracellular vesicles from recurrent implantation failure patients (RIF-EVs) attenuate the growth and implantation potentials of embryos and what are the mechanisms? Summary answer: RIF-EVs inhibited embryonic growth and decreased the trophoblast functions via miR-6131/PAK2 pathway.

What is known already: Recurrent implantation failure (RIF) is characterized by repeated embryo transfers without pregnancy. To date, the etiology of RIF remains poorly understood. Recent evidence indicated that extracellular vesicles (EVs) secreted by endometrial cells, played a crucial role in the implantation by regulating the development and implantation of embryos.

Study design, size, duration: Endometrial cells isolated from endometrial tissues of RIF patients (n=25) and fertile women (n=16) were cultured and modulated via hormones. Endometrial EVs from RIF patients (RIF-EVs) or fertile women (FER-EVs) were isolated from the conditioned medium. The influence of EVs on embryonic development and implantation was investigated by co-culture models of EVs and 2-cell murine embryos or HTR8/SVneo cells, respectively. High-through put sequencing was performed to identify the miRNA profile in the RIF-EVs.

Participants/materials, setting, methods: RIF-EVs and FER-EVs were characterized using western blotting, nanoparticle tracking analysis, and transmission electron microscopy. After co-culture with EVs, embryonic blastocyst rate and hatching rate were calculated. Besides, the proliferation, migration, and invasion of EV-treated trophoblast cells were evaluated by CCK-8, wound healing, and transwell invasion assays. miRNA expression profiles were compared between RIF-EVs and FER-EVs, and the regulatory role of significantly upregulated miR-6131 in RIF-EVs was investigated in the trophoblast cells.

Main results and the role of chance: RIF-EVs and FER-EVs are round bilayer vesicles, ranging mainly at 100 nm and enriched in TSG101, Alix, and CD9. Both RIF-EVs and FER-EVs entered embryonic or trophoblast cytoplasm. The blastocyst rate in the RIF-EV groups was significantly decreased compared to that in the FER-EV groups, at concentrations of 5, 10, and 20 μg/ml. The hatching rate was decreased significantly in embryos treated with 10 or 20 μg/ml RIF-EVs compared to those treated with FER-EVs at the same concentration ($p<0.05$). The proliferation, migration, and invasion of trophoblasts were significantly decreased in the RIF-EV group at 20 μg/mL. A total of 11 differently expressed (fold change >2 and $p< 0.05$) miRNAs were found in the RIF-EVs, and two of them were validated in a larger set of EV samples using RT-PCR. The most significantly different miRNA, 6131, was increased in the RIF-EV-treated HTR8/SVneo cells. The up-regulation of miR-6131 inhibited the growth and invasion of HTR8/SVneo. Bioinformatics coupled with luciferase and western blot assays revealed that PAK2 is a direct target of miR-6131, and the overexpression of PAK2 can rescue the phenotype changes induced by miR-6131 overexpression.

Limitations, reasons for caution: Our study indicated miRNA in the RIF-EVs dysregulating the growth and function of embryonic cells. However, EVs contained a wide spectrum of bioactive molecules, including proteins, mRNAs, and DNA, which may play an important role in the implantation. Further studies are required to investigate the mechanisms.

Wider implications of the findings: This work indicates an important role of EVs from women with RIF in embryonic implantation, potentially providing a novel insight to understand the pathophysiology of RIF.

Trial registration number: not applicable

P-389 The relationship between serum hormone profiles and missed abortion in humans

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Study question: Are circulating profiles of metabolic-related hormones also associated with the missed abortion (MA) in humans?

Summary answer: Serum levels of fatty acid-binding protein-4 (FABP4) and fibroblast growth factor 21 (FGF21) are positively associated with MA.

What is known already: A cluster of endocrine hormones, including FABP4, FGF21, adiponectin, lipocalin-2 (LCN2), exhibit pleiotropic effects on regulating systematic metabolism. Serum levels of them are associated with gestational obesity and diabetes and affect pregnancy outcomes, however, the relationship between their circulating profiles and MA is under-investigated.

Study design, size, duration: 78 patients with MA and 86 healthy pregnant subjects matching on maternal age and body mass index (BMI) were nested from a prospective cohort in the Chinese population.

Participants/materials, setting, methods: Fasting serum samples from all participants were collected to test their serum levels of FGF21, FABP4, adiponectin, and LCN2 by enzyme-linked immunosorbent assay method (ELISA).

Main results and the role of chance: There were no significant differences in circulating profiles of adiponectin and LCN2 between MA patients and healthy pregnant subjects. By contrast, circulating levels of FGF21 and FABP4 were significantly and independently elevated in patients with MA relative to control cases even after adjusting confounding factors (for FGF21: MA: 28.96 ± 2.17 ng/ml; HP: 19.18 ± 1.12 ng/ml, $P< 0.001$, for FABP4: MA: 152.50 ± 9.31 pg/ml; HP: 90.86 ± 4.14 pg/ml, $P< 0.001$). Linear regression analysis showed, FGF21 raised every 10 pg/ml contributed to a 24% (95% CI: 15% - 34%) increase in the risk of MA, whereas the OR of FABP4 for the risk of MA was 1.052 (95% CI: 1.022 -1.088). Furthermore, using serum FGF21 level or FABP4 levels discriminated MA from healthy controls with an area under the operating characteristic's curve (AUROC) of 0.81 (95% CI 0.76-0.92) and 0.70 (95% CI 0.62 - 0.78), respectively.

Limitations, reasons for caution: The study is limited by the sample size. In addition, our results were based-on Chinese population, whether it could be observed in other ethics group remain to be investigated. Meanwhile, the cause-effect relationship between increased serum FGF21 level and MA remains to be explored.

Wider implications of the findings: Our data would suggest that serum levels of FGF21 and FABP4 are associated with MA. Moreover, circulating FGF21 levels may serve as a potential diagnostic biomarker for the recognition of M.

Trial registration number: IRB Ref. No.: KY201913

P-390 The intrauterus administration of peripheral blood mononuclear cells increase the pregnancy rates for the patients with advanced maternal age after single euploid embryo transfer

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Study question: Does the intrauterine administration of peripheral blood mononuclear cells (PBMCs) effect the outcome of IVF for patients with advanced maternal age when the euploid embryos after PGT-A are transferred?
Summary answer: The implantation rates were significantly higher after the intrauterine application of PBMCs in patients with advanced maternal age (AMA) before transfer of the euploid embryo.
What is known already: The aneuploidy rates of blastocysts in IVF is in range 45-70% depending on different factors. Besides that, the endometrium plays an important role in achieving optimal outcomes of assisted reproductive technologies. It has been proposed that intrauterine administration of peripheral blood mononuclear cells modulates maternal immune response to favor implantation.
Study design, size, duration: The effect of the intrauterine application of PBMCs to improve the implantation rates in the group of patients with advanced maternal age was studied. Two group of patients (PBMCs-group and non-PBMCs-group_ were formed. Single euploid embryo was transferred for each patient. Participants/materials, setting, methods: The ploidy status of 373 blastocysts from 82 AMA-patients was analyzed by the method of next generation sequencing (NGS). PBMCs were applied for 39 women with the mean age 39.2±3.2 y.o. before embryo transfer (Group 1). For 43 patients with the mean age 38.2±2.1 y.o. single euploid embryo transfers were performed without PBMCs administration (Group 2). Chi-squared test was used for data analysis. The study's protocol was approved by the Center's IRB.
Main results and the role of chance: Totally the rate of euploid embryos was 27.1% (101 blastocysts). In the mentioned study 55.0% of examined blastocysts were aneuploid (205 embryos) and 17.9% of blastocysts were detected as mosaic (67 embryos). Single euploid embryo was transferred in each case in the patients of both experimental groups. The implantation rate was significantly higher in Group 1 with PBMCs application comparing with non-PBMCs experimental Group 2 (38.5% (15 pregnancies) vs. 23.3% (10 pregnancies), df = 1, $\chi 2 = 5.487$, $\chi 2$critical = 3.841, P = 0.020).
Limitations, reasons for caution: The embryo biopsy was performed only for blastocyst with top-quality morphology.
Wider implications of the findings: The implantation rates were significantly higher when the intrauterine application of PBMCs in patients with advanced maternal age before the transfer of the euploid embryo (P = 0.020). The randomized studies to improve our knowledge in immunogenic therapy in reproductive medicine should be performed.
Trial registration number: -

P-391 Role of subcutaneous granulocyte colony-stimulating factor infusion in thin endometrium

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Study question: To assess the role of subcutaneous granulocyte colony-stimulating factor (G-CSF) in thin endometrium cases.
Summary answer: G CSF has beneficial role to improve the endometrium thickness in thin endometrium.
What is known already: Endometrium is very important for embryo implantation and the endometrial thickness is the marker of receptivity of the endometrium.
Study design, size, duration: Study design - Retrospective analysis
Size - 88 infertile females with thin endometrium (< 7 mm) in the age group of 23 to 40 years Duration - one year.
Participants/materials, setting, methods: In the group 1 of 44 females, subcutaneous infusion of G CSF (300 mcg/ml) was added along with other supplements and if lining was not more than 7 mm in 72 hours, then second infusion was given. In the group 2 of 44 females, only estradiol valerate and sildenafil were given.The efficacy of G CSF was evaluated by assessing the endometrium thickness before embryo transfer, pregnancy rates and clinical pregnancy rates.
Main results and the role of chance: There was no difference between the two groups regarding demographic variables, egg reserve, sperm parameters, number of embryos transferred and embryo quality. . The pregnancy rate was 60% (24 out of 40 cases) in the group 1 that was significantly higher than in-group 2 that was 31% (9 out of 29 cases) with p value < 0.0001. The clinical pregnancy rate was also significantly higher in-group 1 (55%) as compared to group 2 (24%) with p value < 0.0001.
Limitations, reasons for caution: Further larger cohort studies are required to explore the subcutaneous role of G CSF in thin endometrium.
Wider implications of the findings: Granulocyte colony-stimulating factor has beneficial role to improve the endometrium thickness in thin endometrium. In most of previous studies, the intrauterine infusion of G CSF was given to improve the uterine lining. This is one of the few studies done that showed subcutaneous role of G CSF in thin endometrium.
Trial registration number: Not applicable

P-392 Clinical outcomes of endometrium receptivity analysis(ERA) testing in patients with repeated IVF failures

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Study question: Is ERA testing different between RIF patients with control group?
Summary answer: In RIF patients, there were more chances of non-receptive endometrium. ERA testing may be helpful for the patients with repeated IVF failure. What is known already: The endometrium receptivity analysis testing might have the ability to detect the implantation window. In repeat implantation failure patients, detecting of precisely implantation window may have some benefits.
Study design, size, duration: This was a single-center retrospective observational study. Two hundred and forty-nine patients who underwent ERA testing following frozen-thawed embryo transfer in our center were including in this study between January 2019 and May 2020.
Participants/materials, setting, methods: 181 patients having unexplained repeated IVF failure (RIF group, at least tow implantation failure) and 68 patients having no experience with embryo transfer (Control group) who underwent ERA testing were including in this study. Both of Patients having a receptive (R) ERA and having a non-receptive (NR) ERA underwent a personalized embryo transfer (pET) on ERA. ERA results and clinical outcomes compared between RIF group and control group were analyzed by Chi-square test.
Main results and the role of chance: The proportion of R/NR results were 33:35 for the RIF group and 118:63 for the Control group, demonstrating the displacement of the window of implantation in patients with RIF. Our results revealed an endometrial factor in 51% RIF patients, which was significantly greater than the Control group 34.8% ($P = 0.02$). Among the patients with NR ERA result, there are not significantly difference in clinical pregnancy rate in the RIF group compared with control group (57.1%. vs. 61.9%). The clinical pregnancy rate of the patients with receptive ERA result also is comparable in both group (70.3% vs. 66.7%).
Limitations, reasons for caution: This is a retrospective, single center study with limited case number. There were may some bias with ERA testing errors.
Wider implications of the findings: In RIF patients, there were more chances of non-receptive endometrium. ERA testing may be helpful for the patients with repeated IVF failure. Larger randomized studies are required to validate these results.
Trial registration number: 18MMHISO70e

P-393 The relationship of cigarette smoking with gestational diabetes. An evaluation of a database of more than nine million deliveries

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Study question: Given the common pathophysiology between type 2 DM (risk of which is increased by smoking) and GDM we sought to assess whether an association between smoking and GDM exists?
Summary answer: After controlling for confounding effects, women who smoke during pregnancy are at an increased risk of developing GDM.
What is known already: Smoking is well associated with type 2 diabetes mellitus (DM) in multiple studies. It has remained unclear whether there is also an association between smoking and GDM as publications report conflicting results. In a meta-analysis of 1,364,468 pregnancies (22,811 smokers) there was no association between cigarette smoking and the risk of GDM. While a study from the Pregnancy Risk Assessment Monitoring System, on 222,408 patients (54,114 smoked during pregnancy) found a higher risk for GDM among smokers.
Study design, size, duration: A retrospective population-based study utilizing data from the Healthcare Cost and Utilization Project—Nationwide Inpatient Sample (HCUP-NIS). A dataset of all deliveries between 2004 and 2014 inclusively, was created. Within this group, all deliveries to women who smoked during pregnancy were identified as part of the study group (n=443,590), and the remaining deliveries were categorized as non smoker births and comprised the reference group (n= 8,653,198).
Participants/materials, setting, methods: The HCUP-NIS is the largest inpatient sample database in the USA, and it is comprised of hospitalizations throughout the country. It provides information relating to 20% of US admissions and represents over 96% of the American population. Multivariate logistic regression analysis, controlling for confounding effects, was conducted to explore associations between smoking and delivery and neonatal outcomes. According to Tri-Council Policy statement (2018), IRB approval was not required, given data was anonymous and publicly available.
Main results and the role of chance: Our study identified 9,096,788 births between 2004-2014, of which 443,590 (4.8%) had a documented diagnosis of maternal smoking. Smokers were more likely to be young (53% vs 37.2% under the age of 35), white (78% vs 51.1%), of lower income (39.1% vs 26.6%), delivered in a rural hospital (28.7% vs 13.2%), suffer from obesity (6.4% vs 3.4%), have pregestational diabetes (1.2% vs 0.9%) and chronic hypertension (2.5% vs 1.8%) and to have undergone a previous caesarean section (17.7% vs 5.9%) (all p value <0.0001, all were controlled for in the logistic regression analysis). An increased risk for GDM among smokers was detected with an adjusted odds ratio (aOR) of 1.10 (95%CI:1.07-1.14 p<0.0001), when controlling for the factors above. A significant higher risk of preterm delivery (aOR1.39, 95%CI:1.35-1.43, p<0.0001), PPROM (aOR 1.52 ,95%CI:1.43-1.62, p< 0.0001), wound complications (aOR1.24,95%CI:1.09-1.41, p<0.0001), and the need for hysterectomy (aOR1.32,95%CI:1.0-1.64,p< 0.0001) among the smokers was found as well.
Limitations, reasons for caution: The limitations of our study are its retrospective nature and the fact that it relies on an administrative database.
Wider implications of the findings: The public health implications of confirming smoking as a risk for GDM are many. This can lead to earlier screening in pregnancy of smokers for GDM. The earlier initiation of interventions could decrease fetal complications and possibly have impact on the life and long-term health of that offspring.
Trial registration number: not applicable

P-394 Intralipid infusion at time of embryo transfer in women with history of recurrent implantation failure: a systematic review and meta-analysis

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Study question: What is the effectiveness of IV Intralipid (IVI) in improving pregnancy rates in women undergoing IVF with history of Recurrent implantation failure (RIF) to improve reproductive outcomes.
Summary answer: The evidence to support the use of IVI at the time of embryo transfer in women with RIF is limited. More RCTs are needed. What is known already: Optimising the implantation process following embryo transfer remains a clinical challenge with 10% of couples undergoing IVF affected by (RIF). Immunotherapy could help to optimise endometrial receptivity and increase the chances for successful conception in women with history of RIF. Intra-venous Intralipid (IVI), a fat-based emulsion of soybean oil, glycerine, phospholipids, egg, and polyunsaturated fatty acids, has been evaluated in several trials as a potential intervention to downregulate the uNK cells and macrophages as well as inhibit the pro-inflammatory mediators including T1 helper cells. Evidence synthesis is needed to evaluate the effectiveness of this intervention.
Study design, size, duration: We performed this systematic review using a prospectively registered protocol (CRD42019148517) and reported in accordance with the PRISMA guidelines. Participants/materials, setting, methods: We searched MEDLINE, EMBASE and CENTRAL for any randomised trials evaluating the use of IVI at the time of embryo transfer in women undergoing assisted conception until September 2020. We extracted data in duplicate and assessed risk of bias using the Cochrane Risk of Bias tools. We meta-analysed data using a random effect model and reported on dichotomous outcomes using risk ratio (RR) and 95% confidence interval (CI).
Main results and the role of chance: We included five randomised trials reporting on 843 women with an overall moderate risk of bias. All trials used 20% IVI solution at the time of embryo transfer compared to normal saline infusion or no intervention (routine care). The IVI group had a higher chance of clinical pregnancy (172 vs 119, RR 1.55, 95%CI 1.16-2.07, I2 44.2%) and live birth (132 vs 73, RR 1.83, 95%CI 1.42-2.35, I2 0%) post treatment compared to no intervention.
Limitations, reasons for caution: Our findings are limited by the small sample size and the variations in treatment protocols and population characteristics.
Wider implications of the findings: Our meta-analysis offers an overview on the value of IVI to help women affected by RIF. Given the limitations and the quality of included trials, adopting the use of IVI a-la-carte to couples undergoing IVF remains immature. IVI should not be offered until larger RCTs demonstrate a persistent benefit.
Trial registration number: CRD42019148517

P-395 Obstetric and perinatal outcomes of pregnancies resulting from fresh versus frozen embryo transfer – a sibling cohort

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Study question: We aimed to compare obstetric and perinatal outcomes between pregnancies conceived by in vitro fertilization (IVF) with fresh embryo transfer and frozen embryo transfer (FET) in the same women.
Summary answer: IVF pregnancies following fresh and FET entailed the same obstetric and perinatal outcomes, when compared in the same women. What is known already: There seems to be a difference in adverse outcomes between pregnancies following fresh and FET, as fresh transfer has repeatedly been associated with a higher risk of preterm birth and small for gestational age neonates, and the FET with preeclampsia and large for gestational age neonates. The overall lower incidence of adverse obstetric outcomes in FET may relate to the transfer of an embryo to a uterine environment in the setting of more physiological estradiol level but may also relate to patient characteristics which allow for freezing and subsequent transfer.
Study design, size, duration: This was a retrospective cohort of 214 deliveries during a 13-year period.
Participants/materials, setting, methods: The study was performed in a tertiary hospital. The cohort included live singleton deliveries (>24 weeks of gestation) and excluded pregnancies following egg donation. Each fresh transfer IVF pregnancy was matched to a FET pregnancy by the same woman (1:1 ratio).

Main results and the role of chance: A total of 107 fresh transfer pregnancies were matched to 107 FET pregnancies, in the same women. Mean maternal age was lower in the fresh transfer group compared to the FET group (30.4 vs. 32.5 years, p<0.001), as was body mass index (BMI) (p=0.001). A higher rate of nulliparity was noted in fresh transfer pregnancies (64.5% vs. 12.1%, p<0.001). Mean birthweight was higher in the FET group (3160 vs. 3081 grams, respectively, p<0.001), although the rates of low birth weight and small for gestational age neonates did not differ between the groups. Preterm deliveries occurred in 10.3% and 9.3% of fresh transfer and FET pregnancies respectively, p=0.79. On multivariate linear regression analysis, the type of embryo transfer - FET or fresh - was not independently associated with birthweight, after adjustment for women's age, nulliparity and BMI.
Limitations, reasons for caution: The study relied on coding in patient files, and thus certain data were missing for analysis, such as paternal identity. In addition, women included had at least two successful IVF pregnancies, and at least one cycle in which embryo freezing was performed. This may confer a selection bias.
Wider implications of the findings: Our study of sibling deliveries after fresh and FET, points to a similar prognosis for the main obstetric and perinatal outcomes. This adds to current research which points to similar development of children following fresh and FET and is reassuring for clinicians consulting patients who are eligible for both options.
Trial registration number: Not applicable

P-396 Preconceptual male and female metabolite profiles are associated with ongoing pregnancy after IVF

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Study question: In men and women undergoing IVF are preconceptual circulating metabolites associated with ongoing pregnancy rates?
Summary answer: Preconceptual serum histidine levels, in both women and men were associated with ongoing pregnancy. Several amino acids and lipoproteins exhibited possible sex-specific associations.
What is known already: Preconceptual maternal health has been associated with pregnancy outcomes after IVF. The extent to which this is because of pre-existing metabolic factors related to infertility and the role of paternal metabolic health is unclear.
Study design, size, duration: Cohort of 398 women and 325 male partners prospectively recruited between 1 April 2017 and 31 March 2019.
Participants/materials, setting, methods: Women and their male partners intending to undergo assisted conception at a University Hospital, had detailed pre-treatment phenotyping including non-fasting serum lipids, lipoprotein subclasses, and low-molecular weight metabolites (including amino acids, glycolysis and inflammatory markers) (155 metabolites) quantified by NMR spectroscopy. Multivariable linear and logistic regression were used to examine the associations of pre-treatment serum metabolic profiles, with ongoing pregnancy at 20 weeks gestation with adjustment for confounders.
Main results and the role of chance: 392 women and 322 men proceeded to IVF treatment, with an overall ongoing pregnancy rate of 47.2% (95% CI 0.42, 0.52) per cycle started and a multiple pregnancy rate of 1.1% (95%CI 0.0, 0.04). In both females and males in confounder adjusted analyses histidine was associated with the chance of ongoing pregnancy, with similar magnitudes in each parent (OR 1.28 (95% CI 1.03, 1.60) per one standard deviation (SD) increase for males and OR 1.26 (95% CI 0.99, 1.60) per one SD increase for females). In females Alanine (OR=1.31 (1.05, 1.64)), Isoleucine (OR=1.28 (1.02, 1.61)) and Leucine (OR=1.24 (0.99, 1.55)) had a positive association with ongoing pregnancy, while in males, pyruvate (OR=1.30 (1.02,1.66)) exhibited a positive association with ongoing pregnancy. In both parents, associations of lipids, lipoproteins sub-particles and fatty acids with pregnancy were closer to the null.
Limitations, reasons for caution: Suggestive parental differences could be due to chance. Patients were relatively homogenous undertaking their first IVF cycle and the results may not be generalisable to other clinical populations.
Wider implications of the findings: This study provides data on a range of metabolic pathways and their association with ongoing pregnancy following IVF. The identification of potentially relevant clinical effect sizes in both men and women warrants further exploration.
Trial registration number: not applicable

P-397 Threatened Miscarriage and increase in Perinatal Morbidity

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Study question: Are women presenting with bleeding in the first trimester of pregnancy at a higher risk for perinatal complications later in pregnancy?
Summary answer: Women presenting with bleeding in the first trimester of pregnancy are more likely to experience perinatal and neonatal morbidity in pregnancy.
What is known already: Observational studies and a previously reported systematic review showed that women who experienced threatened miscarriage are more likely to have still birth, intra uterine growth restriction (IUGR), low birth weight, pre-eclampsia, placental abruption, placenta previa, preterm labour, preterm prelabour rupture of membrane, neonatal asphyxia and congenital anomalies in pregnancy. However, the evidence has been inconclusive and currently the women who experience threatened miscarriage receive low risk care.
Study design, size, duration: This was a prospective cohort study conducted on 298 women with threatened miscarriage (Cohort A) and 107 asymptomatic women (Cohort B). The women were recruited over a period of 18 months and were followed up for 9 months until delivery.
Participants/materials, setting, methods: Cohort A were women who presented with bleeding in the early pregnancy assessment unit and had a confirmed heartbeat on ultrasound scan between 6 weeks and 11+6 weeks of pregnancy and cohort B were women who were asymptomatic and booked with the community midwives as low risk. Both groups of women were followed up prospectively until delivery and data were collected on any perinatal outcomes and complications for both mother and the neonate.
Main results and the role of chance: The analysis showed that women who had bleeding in early pregnancy were more likely to have preterm delivery (RR 95% CI; 2.98 (1.07 – 8.27)); IUGR (unable to calculate the RR, as none of the women who continued their pregnancies beyond 24 weeks of gestation, developed IUGR in the asymptomatic control cohort. Nonetheless, IUGR occurred more frequently in the threatened miscarriage cohort than the asymptomatic cohort (P-value 0.02)); LBW (RR 95% CI; 6.14 (1.49 – 25.19), neonatal asphyxia (unable to calculate the RR, as none of the babies who were born to women in the asymptomatic control cohort develop neonatal asphyxia. Nonetheless, neonatal asphyxia occurred more frequently in the threatened miscarriage cohort than the asymptomatic cohort (P-value 0.02)). Preterm prelabour rupture of membrane was not significant with a P-value of 0.07.
Limitations, reasons for caution: The major limitation of this study was lower sample size and hence due to the rarity of many of the perinatal and neonatal outcomes, we were unable to calculate the relative risk.
Wider implications of the findings: Current study agrees with the existing literature and reaffirms the association of perinatal and neonatal morbidities with threatened miscarriage and this group of women need to be managed as high-risk group antenatally.
Trial registration number: not applicable

P-398 Decidualization inhibits the expression of CXCR3-binding chemokines by human decidual stromal cells. Role in maternal-fetal immune tolerance

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Study question: We aimed to analyze the effects of decidualization on the expression of chemokines that attract abortogenic T cells by human DSCs.

Summary answer: Decidualization inhibits the expression of chemokines that attract Th1 and Tc1 cells by DSCs, thereby preventing the arrival of abortogenic T cells into the decidua.

What is known already: Decidual stromal cells (DSCs) are the most abundant cells in the human decidua, the tissue that constitutes the maternal component of the placenta. Numerous evidences confirm that DSCs play a key role in maternal-fetal immune tolerance. In normal pregnancy, DSCs undergo a process of differentiation (decidualization) under the effect of progesterone and other pregnancy hormones. Decidualized DSCs become rounded and secrete prolactin, IL-15 and other factors. In the mouse, it has been observed that during pregnancy, DSCs inhibit the expression of chemokines that attract abortogenic Th1 and Tc1 cells from blood to the decidua.

Study design, size, duration: We compared the expression of CXCR3-binding chemokines by undifferentiated and decidualized human DSCs. We also compared the capacity of these cells to attract activated Th1 and Tc1 cells in vitro. Ten DSC lines were obtained from elective vaginal terminations of first-trimester pregnancies (6-11 weeks). Donors were healthy women aged 20-30 years. Informed consent was obtained from each donor. This study was approved by the Research and Ethics Committee of the University of Granada.

Participants/materials, setting, methods: Decidual stromal cell lines were established as previously described. These lines were decidualized with progesterone and cAMP in vitro. The expression of chemokines by these cells was studied by RT-PCR. Peripheral blood lymphocytes were activated with PHA, anti-CD28 and IL-2. As a consequence of this activation, CXCR3+ Th1 and Tc1 cells were produced. We used a migration assay in Transwell chambers to study the capacity of DSCs to attract these activated T cells.

Main results and the role of chance: We observed that those chemokines that bind to CXCR3, a chemokine receptor detected in activated Th1 and Tc1 cells, were not expressed by either undifferentiated and decidualized DSCs (CXCL9) or their expression was inhibited in decidualized DSCs (CXCL10 $P<0.01$, CXCL11 $P<0.05$). We found that conditioned media of undifferentiated DSCs decreased the migration of CXCR3+ activated T cells (Th1 and Tc1 cells) ($P<0.05$), and this effect was even stronger with conditioned media of decidialized DSCs $P<0.001$). These results demonstrated that decidualization of DSCs during pregnancy inhibits the expression of chemokines that attract Th1 and Tc1 cells by DSCs, thereby preventing the arrival of abortogenic T cells into the decidua.

Limitations, reasons for caution: This is an in vitro study due to the impossibility of performing an in vivo study in humans for ethical reasons.

Wider implications of the findings: Several publications have shown that DSCs have a therapeutic effect in various Th1-associated diseases. Our results explain this effect and suggest the extension of the use of these cells in the treatment of this type of diseases.

Trial registration number: not applicable

P-399 Temporal dynamics of an IVF/ICSI success prediction test based on the vaginal microbiome

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Study question: What is the influence of time on the vaginal microbiome-based prediction of IVF/ICSI success?

Summary answer: Time influences the vaginal microbiome-based prediction of IVF/ICSI success.

What is known already: The association between the microbiome of the lower female reproductive tract and subfertility is discussed extensively suggesting its importance for fertility and fertility treatment. Using a modified next generation sequencing technique, an assay of the vaginal microbiome that predicts the pregnancy chances before starting the IVF/ICSI procedure has been developed and validated (1) displaying profiles associated with a low, medium and high chance of implantation. The vaginal microbiome is already known to change over time (2). However, it remains unclear to what extent spontaneous improvement from a low score can occur and over what time period.

Study design, size, duration: To investigate the spontaneous reversal capacity and associated time period of a low score microbiome profile in IVF-ICSI patients, an observational prospective cohort study of 77 women was performed using the ReceptIVFity assay. Women with medium or high profiles were encouraged to proceed with their ART treatment, whereas women with a low profile were suggested to delay the treatment for 1 month until a subsequent swab was taken with a maximum of 4 repeats.

Participants/materials, setting, methods: The study was carried out in a University based single center setting. Ethical approval was obtained (6259R MPG§23b). Patients between 24 and 41 years of age were included when eligible for their first, second or third IVF or IVF-ICSI attempt. Exclusion criteria were: antibiotic treatment in the 3 months prior to the test, women who have started with hormone treatment in the last 2 months in the context of ovarian stimulation, or downregulation of endometriosis.

Main results and the role of chance: Of the 77 patients included, 53 had a high or medium profile and proceeded with their treatment. 24 had a low profile and were supposed to delay the treatment in favor of a subsequent test. The low profile patients were followed up as indicated in the study description. Unfortunately, 11 of the 24 low score patients dropped out of the study. This relatively high number can only in parts be explained by unswayable medical reasons as no fertilization or embryo arrest but a comparable number of patients dropped out most likely due to Corona restrictions or Corona-related anxiety reasons. In the low score group, 1 month after the initial test, 12 patients repeated the swab; 4 remained low (33,33%), whereas 8 shifted to the medium or high (66,67%) groups. After 2 months, 4 patients had another test; 1 remained low (25%), 3 shifted to medium and high (75%). Therewith, in two months' time 91,7% shifted from low to a better (medium/high) profile. So far, only 1 patient of the initial lows remained low for 5 months. The 12 shifters had a clinical pregnancy rate of 40% after the first embryo transfer after changing the microbiome profile from low to medium/high.

Limitations, reasons for caution: The results described were generated from a smaller group than intended initially due to a relative high dropout rate for no medical reasons.

Wider implications of the findings: Patients suffering from infertility have a clinical benefit from performing a ReceptIVFity test before ART treatment and to delay treatment, when the result is low, since the spontaneous conversion time to a better profile, and therewith a higher pregnancy chance, occurred within 2 month in almost all patients.

Trial registration number: 2018124928

P-400 Endometrial changes in estrogen and progesterone receptor expression during implantation in an oocyte donation program

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Study question: Which are the endometrial changes during implantation in assisted reproduction techniques?

Summary answer: Synchronization between blastocyst development and the acquisition of endometrial receptivity is a prerequisite for the success of *in vitro* fertilisation (IVF).

What is known already: Implantation is the final and most important stage of embryogenesis and is of paramount importance in achieving a successful pregnancy. Progesterone and estrogen are steroid hormones responsible for the regulation of the implantation window and the current study hypothesised that their receptors may be implicated in women undergoing oocyte donation.

Implantation is directly dependent on the synchronization of the fertilized egg's progression into a blastocyst and the specific differentiation of the endometrium through molecular and cellular changes regulated by agents with an endocrine, paracrine or autocrine activity.

Study design, size, duration: The study was conducted at the 1st Dept. of OB-GYN, Centre for Human Reproduction of the Aristotle University of Thessaloniki, 'Papageorgiou' General Hospital and the 'Biogenesis' Assisted Reproduction Centre, (both in Thessaloniki, Greece).

Participants/materials, setting, methods: The participants recruited for this prospective study included 15 oocyte donors (age range, 25-32 years; mean age, 28.9±2.89) undergoing IVF treatment. The inclusion criteria were white race, no uterine-ovarian pathology, age <35 years and no prior known medical pathology. All donors had undergone extensive preoperative work-up, which included common blood tests, karyotyping, specific test for cystic fibrosis and pap smear. All donors were non-smokers and had given their informed consent.

Main results and the role of chance: Both ERα and PR-B were expressed abundantly on both days (0 and 5; Fig. 1B). The ERα nodal staining percentage on day 0 was age-related, with patients aged <30 years showing 100% staining and those aged >30 years showing 90% staining (Mann-Whitney U test; P=0.014; Fig. 2A)Both steroid hormone receptors showed significant variation between days 0 and 5, both in the nodal and stromal preparations. According to Wilcoxon signed-rank test; for ER (nodes % and stromal %) Day 0/5, P=0.0001; for PR (nodes % and stromal %) Day 0/5, P=0.0001 and P=0.035, respectively; for ER (Grade nodes and stromal) Day 0/5, P=0.0001; and for PR (Grade nodes and stromal) Day 0/5, P=0.0001 and P=0.016, respectively (Fig. 2B and andC;C; Table I).

Limitations, reasons for caution: Immunohistochemistry is less quantitative than western blotting. Alternatively, ELISA or a gene expression assessment of both receptors using RT-qPCR could have been conducted. However, due to ethical restrictions, sufficient tissue for protein extraction could not be obtained in order to pursue this further.

Wider implications of the findings: It was shown herein that both ER-a and PR-B were expressed abundantly on days 0 and 5, showing significant variation in the nodal and stromal preparations. Age appeared to be a critical factor, since ER-a nodal staining showed higher values in the age group of oocyte donors <30 years old.

Trial registration number: 15

P-401 Frozen-thawed embryo-transfer adjuvant therapy: one size DOES NOT fit all

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Study question: Does adjuvant therapy after frozen-thawed embryo-transfer (FRET) with CardioAspirin and Prednisone enhance clinical pregnancy rate (CPR) and live birth rate (LBR)?

Summary answer: Adjuvant therapy enhanced CPR and LBR in study-group. A significant correlation was found confronting blastocyst FRET in study-group versus controls.

What is known already: Embryo implantation is a rate-limiting step of FRET cycles. It's a complex process resulting from a balance between inflammation pathways and maternal immune tolerance. Low-dose aspirin unlocks Prostaglandin-F2 synthesis by Cyclooxygenase-1, thus increasing uterine vascular permeability and attachment reaction while reducing vasoconstriction. Pregnancy results from a balance between helper and regulatory T-cells (Treg), the latter protect the embryo from maternal immune attack. Treg cells' immunosuppressive function is pivotal in pregnancy establishment. Prednisone increases the proportion of Treg cells thus inhibiting inflammation. Many therapy schedules for implantation enhancement are currently used worldwide, although there is no consistent shared evidence.

Study design, size, duration: Retrospective cohort-control study including 237 subjects who underwent FRET after artificial endometrial-preparation from January 2018 to March 2020. Estrogenic stimulation was either oral or transdermic. The study-group received luteal support (vaginal Progesterone 600 mg/die) and adjuvant therapy (CardioAspirin and Prednisone 25-5 mg); the control-group received luteal support only. Pregnancy test (PT) was scheduled 10-14 days post-transfer (blastocysts or cleavage stage embryos). Second PT and ultrasound were performed 7 days later if the first was positive.

Participants/materials, setting, methods: Patients referred to Padua University Hospital's Human Reproduction Pathophysiology Unit. Exclusion criteria: >50/<18 years, fresh embryo-transfer cycles, oocyte-thawing cycles, natural/natural-modified cycles. Male factor was the prevalent fertility issue. Single embryo-transfer was performed in both groups. Mean endometrial thickness was 9 mm trilaminar in both groups. Statistical analysis were carried out using JMP Pro 14 software. Categorical variables were analyzed using Chi-square test or Fisher's exact test where appropriate.

Main results and the role of chance: In the study-group, 87 subjects were given luteal support and adjuvant therapy, while in the control-group, 150 subjects received luteal support only. Groups were homogeneous for age, number of embryos transferred, endometrial thickness, endometrial features (trilaminarity) and fertilization tecniques (108 IVF/ 127 ICSI). CPR and LBR were significantly higher in the study-group. CPR was 31.4% in study-group versus 14.8% in controls (p=0.002), LBR was 27.4% in study-group versus 11.6% in controls (p=0.002). Since heterogeneity between groups was found regarding the type of embryo transferred (55.3% cleavage-stage versus 44.7% blastocyst, p<0.01), the groups were split analyzed basing upon the type if embryo transferred. In the cleavage-stage FRET condition no relevant correlation was found between groups. However in blastocyst-FRET group CPR (34.5% study-group versus 18% controls, p=0.04) and LBR (30.9% study-group versus 12% controls, p=0.017) were significantly higher in the study-group, thus showing that adjuvant therapy could improve CPR and LBR.

Limitations, reasons for caution: Limited sample size negatively impacts the study's power. It would be appropriate to expand the sample to obtain more reliable results.

Wider implications of the findings: Although no unanimous consent exists for tout-court adjuvant therapy administration, scientific literature shows that such therapy can help patients with repeated implantation failures or anti-nuclear-antibodies positivity. Assuming that a single-therapy-regimen could perfectly fit all patients is not realistic. We have to move towards patient-tailored adjuvant therapy thinking.

Trial registration number: Not Applicable

P-402 Single embryo transfer in the Spanish public health system

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Study question: Are there significant advantages to transferring two embryos over transferring a single embryo in assisted reproduction?

Summary answer: The transfer of two embryos increases the number of miscarriages and multiple gestations without significantly increasing the pregnancy and live newborn rates.

What is known already: One of the most frequent complications in women who undergo in vitro fertilization treatments is multiple pregnancy. The objective of assisted reproductive techniques is to achieve a healthy and alive newborn. Therefore, for some years now, the embryo with the greatest implantation capacity to transfer has been chosen and, although the trend is to transfer a single embryo, the transfer rate of two embryos is still very high (60.3% according to the 2017 National Registry of the Spanish Fertility Society).

Study design, size, duration: Retrospective study in which 274 transfers made in the Assisted Reproduction Unit of the Valme University Hospital (Seville, Spain) during 19 months (November 2018 to May 2020) were analyzed. The transfers were divided into two groups: eSET (elective single embryo transfer) and DET (double embryo transfer).

Participants/materials, setting, methods: The rates of clinical pregnancy, multiple pregnancy, live newborn and abortion were evaluated in both groups. The comparison of the results was performed using Pearson's Chi-square test (SPSS Statistics software). Statistical significance was defined as p<0.05.

Main results and the role of chance: Of the 274 embryo transfers performed, 195 were eSET (71.2%) and 79 DET (28.8%). The gestation rate in the

eSET group was 43.1% while in the DET group it was 45.6%, with no statistically significant differences (p = 0.707). There was no multiple pregnancy in the eSET group while the multiple pregnancy rate in the DET group was 33.3%, with statistically significant differences (p <0.001). The abortion rate in the eSET group was 5.6% while in the DET group it was 13.9%, with statistically significant differences (p = 0.023). The live birth rate in the eSET group was 35.9% while in the DET group it was 31.6%, with no statistically significant differences (p = 0.504).

According to the results obtained in our Assisted Reproduction Unit, although the transfer of two embryos achieves a higher gestation rate, the difference is not statistically significant, so it should be reserved for very specific cases. The transfer of two embryos, on the other hand, increases the number of abortions (13.9%) and, above all, the number of multiple pregnancies (33.3%) that can lead to perinatal complications and mortality.

Limitations, reasons for caution: In this study the age of the patients and the cause of infertility were not taken into account. These two factors could influence the results obtained.

Wider implications of the findings: The results of this study support the elective transfer of a single embryo, as we are doing in our Assisted Reproduction Unit in 71.2% of cases, a figure much higher than the 37.2% of eSET nationwide

Trial registration number: not applicable

P-403 Sodium tungstate increases embryo adhesion through a direct effect on endometrial cells

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Study question: Does sodium tungstate treatment induce a change in endometrial cells' capacity to implant trophoblasts?

Summary answer: Administration of sodium tungstate to endometrial cells increases trophoblast adhesion.

What is known already: Sodium tungstate (ST) has shown its capacity to modulate the activity of cytokines, such as leptin, an activator of an obligatory signalling cascade in the embryo-implantation process. STAT3, a signal transducer molecule critical for the embryo implantation process, is also known to be activated by ST. Still, ST's effect on implantation using biological systems has never been studied. Embryo implantation process and endometrium roles are complicated to study *in vivo* due to a lack of animal models and appropriate techniques. *In vitro* techniques using immortalised cell lines allows a first approach to study early implantation stages, such as embryo adhesion.

Study design, size, duration: An *in vitro* study was carried out using a human endometrial carcinoma cell line (HEC-1-A) treated with sodium tungstate for 24 and 48h, and choriocarcinoma cell spheroids (JAr). Different times of treatment and concentrations were studied. Each experiment was performed in triplicate.

Participants/materials, setting, methods: Confluent endometrial HEC-1-A cultures were treated with ST at concentrations (0-150mM) and withaferin A (1mM), negative control for embryo adhesion. After the treatment period, HEC-1-A cultures were washed with ST-free culture medium to eliminate ST. Immediately, 15 JAr trophoblast spheroids were added to cultures and coincubated with gentle agitation for 30, 60 and 90 minutes. An inverted light microscope was used to count adhered and floating spheroids, and determine the trophoblast adherence ratio.

Main results and the role of chance: HEC-1-A cells treated with ST showed normal morphology and growth at all doses except 150mM. At the highest dose tested, the cells' culture was still viable (negative blue trypan staining) and maintained morphology, but the adhesion to the plate surface was affected. Doses from 0.15 to 15mM were used to perform adhesion assays.

HEC-1-A cells treated with ST for 24h showed an increased capacity to adhere JAr trophoblast spheroids. Adhesion rates reached significant differences at doses of 1.5 and 15mM after 60 and 90 minutes of coincubation. After 90 minutes, untreated cells reached 32.8% adhesion rate, while 1.5 and 15mM ST-treated cells reached 54.6% and 53.4% respectively (p<0.05 ST vs untreated). Thus, the increment of trophoblast adhesion rate induced by ST reached 66%. Lower adhesion rates were observed after 60 minutes of coincubation but were also significant with a relative increase of 49.1% at 1.5mM and 50.5% at 1.5mM when compared with untreated cells (p<0.05)

Longer treatments (48h) showed similar trends to 24h-treatments, but with a lower extent of ST effect on HEC-1-A receptivity. Maximum adhesion rates were also observed at 90 minutes of coincubation and 1.5 and 15mM doses. The Mean adhesion rate increase was >40% with both doses. Limitations, reasons for caution: The current study is the first approach to evaluate sodium tungstate effect on endometrium using an *in vitro* model. Future research using *in vivo* models should be performed to assess sodium tungstate effect on endometrium receptivity and its potential as a fertility treatment.

Wider implications of the findings: We conclude that the direct effect of sodium tungstate on endometrial cells increases embryo adhesion rate. These results open a new research line to a potential treatment in human reproduction management with sodium tungstate to solve the unmet need of inducing embryo implantation.

Trial registration number: not applicable

P-404 Low serum progesterone on the day of frozen blastocyst transfer is associated with a diminished ongoing pregnancy rate in hormonal replacement therapy cycles

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Study question: Is there a relationship between progesterone levels on the day of frozen blastocyst transfer and ongoing pregnancy rate (OPR), in hormonal replacement therapy (HRT) cycles?

Summary answer: Women undergoing HRT-frozen embryo transfer with progesterone levels≤9.76ng/ml on the day of blastocyst transfer had a significantly lower OPR than those with progesterone levels>9.76 ng/ml.

What is known already: The importance of serum progesterone levels around the time of frozen embryo transfer (FET) is a burning issue, in view of the growing number of FET worldwide. However, the optimal range of serum progesterone levels is not clearly determined and discrepancies arise from the current literature. Study design, size, duration: Observational cohort study with 915 patients undergoing HRT-FET at a tertiary care university hospital, between January 2019 and March 2020.

Participants/materials, setting, methods: Patients undergoing single autologous blastocyst FET under HRT using exogenous estradiol and vaginal micronized progesterone for endometrial preparation. Women were only included once during the study period. The serum progesterone level was measured in the morning of the FET, in a single laboratory. The primary endpoint was OPR beyond pregnancy week 12. Statistical analysis was conducted using univariate and multivariate logistic regression models.

Main results and the role of chance: Mean serum progesterone level on the day of FET was 12.90 ± 4.89 ng/ml). The OPR was 35.5% (325/915) in the overall population. Patients with a progesterone level ≤ 25th percentile (≤9.76ng/ml) had a significantly lower OPR and a higher miscarriage rate (MR) compared with women with progesterone level over Centile 25 (29.6% versus 37.4%; p=0.033 and 34.8% versus 21.3%; p=0.008, respectively). After adjustment for the potential confounders in a multivariate analysis, a serum progesterone level ≤ 9.76 ng/ml on the day of FETand FET of a Day 6-blastocyst (versus Day 5-blastocyst) were found as independent risks factor of lower OPR.

Limitations, reasons for caution: The main limitation of our study is linked to its observational design. Extrapolation of our results to other laboratories, or other routes and/or doses of administering progesterone also needs to be validated.

Wider implications of the findings: This study suggests that a minimum serum progesterone level is needed to optimize reproductive outcomes in autologous blastocyst FET, in HRT-cycles. Further studies are needed to evaluate if modifications of progesterone routes and/or doses may improve pregnancy chances, in an approach to individualize the management of ART patients.

Trial registration number: NA

P-405 The diagnosis and management of heterotopic intramural pregnancy after in vitro fertilization-embryo transfer: six-case series

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Study question: What are the ultrasonic characteristics of heterotopic intramural pregnancy (HIMP)? How to manage and what about the clinical outcomes of HIMP?
Summary answer: Expectant management may be a considerable choice for an non-viable intramural pregnancy (IMP). Most intrauteine pregnancies (IUPs) of HIMPs seems to have good outcomes.
What is known already: Heterotopic pregnancy (HP) post in vitro fertilization is very rare in infertility women, with a prevalence of 0.04%. HIMP is one of the rarest types of HP, where one gestational sac (GS) is embedded within the endometrial cavity and the other one GS is implanted in the myometriun. HIMP was firstly and only described by Jiangtao Lyu *et al*. in 2018. So far, little is known about its natural history and ultrasonic imaging characteristics. And there is no consensus regarding the ultrasound diagnosis and clinical management for HIMP due to few evidence-based medicine records.
Study design, size, duration: A retrospective observational study was conducted of 6 infertile women who obtained a HIMP through in vitro fertilization-embryo transfer (IVF-ET) between January 2009 and December 2019 at our reproductive centre.
Participants/materials, setting, methods: Six infertile women conceived a HIMP via IVF-ET were retrospectively retrieved between January 2009 and December 2019 at the Reproductive and Genetic Hospital of CITIC-Xiangya (Changsha City, China). The ultrasound diagnosis, clinical management and pregnancy outcome of these cases were analysed. The ultrasound findings, therapeutic methods and clinical outcomes were obtained from the hospital's electronic medical records. This study was approved by the local ethics committee. Main results and the role of chance: Six women with HIMPs were retrospectively analysed. Among them, 5 cases were revealed by ultrasound scans; however, one case was misdiagnosed. The diagnostic accuracy was 83.3%.

Five cases of HIMP were diagnosed at initial scan. The diagnostic time ranged from 22 to 38 days after ET (5+6 - 7+6 weeks). Among them, an intramural GS was observed in all 5 cases; embryonic cardiac activity (ECA) was detected in one case by the followed-up scans; there was a yolk sac only in one case; an empty GS was noted in 3 cases. An IUP was revealed in all 6 cases, and ECA was observed in 5 cases at the initial diagnosis or later. A GS with a yolk sac only was showed in one case.

Among the 5 diagnostic women, one case with a live IMP was treated with laparoscopy at 8+1 weeks, 4 cases were managed expectantly. Of them, the IUPs of 4 cases delivered live infants and one case managed expectantly experienced miscarriage. In one case, IMP was misdiagnosed as interstitial pregnancy at day-28 scan. Exploratory laparoscopy and foetal reduction were performed at 8+2 weeks. Laparoscopy confirmed an IMP and the retained IUP delivered a live infant.
Limitations, reasons for caution: The case numbers are too few to draw any objective conclusions, because of the extreme rarity of HIMP. Thus, a further multi-centre larger prospective study will help to confidently illustrate the clinical significance, and effective and appropriate management method for women with a HIMP.
Wider implications of the findings: Our study showed that HIMP may not be as rare as previously reported. Increased awareness of this condition, better comprehension of the diagnostic criteria and improved resolution of ultrasound equipment may result in more frequent and accurate detection of HIMP, which will be helpful for early management to preserve IUP.
Trial registration number: Not applicable.

P-406 Placental histopathology is different in specific subsets of ICSI singleton pregnancies with programmed cycles : a prospective study

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Study question: Does embryo vitrification or donor oocytes (DO) alter the histopathology of the placenta in ICSI singleton pregnancies with similar endometrial preparation?
Summary answer: Placentas from programmed cycles had significantly more immune/idiopathic-inflammation with vitrified-thawed embryos versus fresh transfer and significantly more maternal vascular-malperfusion(MVM) in DO versus autologous oocyte(AO) pregnancies.
What is known already: DO pregnancies and frozen embryo transfer(FET) pregnancies with programmed cycles are associated with hypertensive complications. As these complications are linked with abnormal placentation, comparing the placental histopathology in these pregnancies may point to a causative association.

Studies of placental histopathology in DO in comparison to AO pregnancies show a dysregulated immune process and vasculopathy. The hormonal milieu during implantation remains an important confounder.

Placental histopathology in fresh/ frozen cycles has recently shown variable results. To isolate the effect of embryo vitrification on placental histopathology, the donor oocyte model can provide valuable data, which till now is scarcely available.
Study design, size, duration: A prospective cohort study conducted in a tertiary center from 2018-2020.

Placental histopathology, pregnancy-outcomes were studied in 116 ICSI singleton pregnancies≥28 weeks.

Group1-Pregnancies with DO, by FET(n=32) and freshET(n=34) were compared to study the effect of embryo-vitrification.

Group2-Pregnancies by DO FET(n= 32) were compared to AO FET(n= 50) to study the effect of DO.

All patients had ICSI, cleavage embryo-transfer, programmed cycles and delivered at the same institute.

The placentas were examined by pathologists (blinded to the ET type).
Participants/materials, setting, methods: 116 singleton pregnancies were followed for hypertensive disorders of pregnancy (HDP), preterm delivery(PTD<37weeks) and low birth-weight (LBW<2.5kg).

Placentas were examined for cord mal-insertions

Placental histopathology lesions were classified into 4 groups according to 'Amsterdam criteria' infectious-inflammatory, immune/ idiopathic-inflammatory, MVM, fetal vascular malperfusion (FVM).

Chi-square and t-tests were used to compare outcomes across groups. Adjusted odds ratio were calculated using logistic regression. Statistical significance set at $P < .05$, two-tailed.
Main results and the role of chance: No patient had a history of chronic hypertension/smoking. **Group 1** Patients conceived by DO, with FET and freshET were comparable with regards to age (34.1 vs 36.4years, *P*=.07),BMI(26.7 vs27.1 kg/m2,*P*=.6),nulliparity(81%vs82%,*P*=.9) HDP(25%vs29.4%,*P*=0.69),birthweight(2.48 vs 2.47kg,*P*=.93) LBW(31.3%vs41.2%,*P*=.41)respectively

PTD was significantly less in donor FET versus donor freshET (6.3%vs47.1%*P*=.0002) Placental weight and cord mal-insertions were comparable for FET vs freshET (466 vs 486gms *P*=.03 12.5%vs23.5% *P*=.25)respectively. Amongst the placental histopathology lesions, immune/ idiopathic-inflammatory lesions were significantly more in the FET vs freshET group (37.5% vs 11.8%,*P*=.02)The other lesions were comparable infectious-inflammatory(6.3%vs17.6%,*P*=.16), MVM(75%vs58.8%, *P*=.16),FVM(18.8%vs17.6%,*P*=.9)

Group 2 Patients conceived by DO compared to AO by FET were significantly older and had a higher BMI (34.1vs31.7years,*P*=.02 ,26.7vs25.5 kg/m2,*P*=.002) respectively.

Nulliparity was comparable(81%vs92%,*P*=.15) Birth weight was significantly less in DO vs AO(2.4vs2.7kg,*P*=.02) HDP and LBW were significantly more in DO vs AO(25%vs8% ,*P*=.03, 31.3%vs 8%,*P*=.007),respectively. PTD was comparable(6.3%vs8.0%,*P*=.77). Placental weight was significantly less in DO vs AO (466 vs 513gms,*P*=.03) cord mal-insertions were comparable(12.5% vs 24%,*P*=.2) The MVM lesions were significantly more in the DO group compared to AO(75% vs 40%,*P*=.002)

The difference remained after adjusting for age/BMI/HDP (AOR 4.31;95% CI 1.24-14.8;*P*=.02). The rest of placental lesions were comparable in DO vs

AO, infectious-inflammatory lesions(6.3%vs16%,*P*=.19) immune/idiopathic-inflammatory lesions(37.5%vs28%,*P*=.37) FVM(18.8% vs 12%,*P*=.4)respectively.
Limitations, reasons for caution: These findings are based on a small number of patients. The results observed need to be confirmed using a larger study sample.
Wider implications of the findings: Placentas in pregnancies by embryo-vitrification, in a DO-model, had significantly more immune/idiopathic-inflammation, the cause/significance of this needs to be explored. Placentas in DO-pregnancies had significantly more MVM-lesions and increased risk of HDP, emphasizing the clinical/histopathological link of DO with HDP and the need for counselling/preventive strategies for HDP in DO-pregnancie.
Trial registration number: not applicable

P-407 Early pregnancy in the Emergency Department; presentation, management, outcome and the effect of COVID-19

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Study question: Has the COVID-19 pandemic and public health guidance impacted referrals, outcome and management of early pregnancy in the emergency room?
Summary answer: COVID-19 changed the way in which women sought guidance and accessed services in early pregnancy.
What is known already: Spontaneous miscarriage is the most common complication of pregnancy1. Experiencing an early pregnancy loss is often an unexpected and difficult time that can be physically traumatising2. A previous study looking at the experience of a miscarriage from both the female and male point of view identified that long waiting times surrounded by other pregnant women in the Emergency Department (ED) was particularly difficult part of the experience2. The COVID-19 pandemic had a significant impact on both hospital and community services. Public health advice also changed the way women accessed healthcare.
Study design, size, duration: Retrospective audit was performed over two six-month periods – July to December 2019 and March to August 2020. Two groups of data were collected; women who contacted the ED with concerns related to early pregnancy (under thirteen weeks gestation) and those who attended the ED with the same complaints. Information was cross referenced to see how many women contacted the ED prior to arrival and what, if any advice was given.
Participants/materials, setting, methods: All women under thirteen weeks gestation with a complaint of bleeding per vaginum (PV) or pain related to early pregnancy who presented to the ED in a large tertiary maternity unit were included in the audit. All women meeting the same criteria who contacted the ED by telephone were also included.
Main results and the role of chance: Over the twelve months of data collection, 1274 women had their first visit to the ED. There were 270 further visits within the early pregnancy period recorded for the same cohort of women. Additionally, 1452 phone calls were recorded. There was a 38% (n=293) decrease in women attending the emergency room in early pregnancy in 2020 during the first wave of COVID-19. There was a 16% (n=110) increase in women contacting the ED for advice in early pregnancy in the same period in 2020. Women were more like likely to have been referred to the ED by their General Practitioner (GP) (OR 0.62, 95%CI 0.48-0.80) and to have phoned in advance of arrival (OR 1.55, 95%CI 1.17-2.04) in 2020. They were also more likely to have already had a previous ultrasound scan in the current pregnancy (OR 0.64, 95%CI 0.48-0.93). There was a significantly shorter waiting time for an appointment in the early pregnancy clinic in 2020 compared with 2019 (3.5 days versus 2.4 days, p=0.002). There was no change in the number of women admitted (OR 1.19, 95%CI 0.81-1.74).
Limitations, reasons for caution: Single centre audit. Pregnancies only followed to booking visit/dating scan and outcome noted at that stage.
Wider implications of the findings: The COVID-19 pandemic highlighted the need for more education around early pregnancy. Easily accessible information about local early pregnancy services gives women autonomy. Phone triage allowing referral of women to appropriate services, reduces ED visits. Standard training in early pregnancy ultrasound could reduce follow up referrals and admission rates.
Trial registration number: not applicable

P-408 Inadequate increase in Tim-3 on peripheral NK cells after blastocyst transfer is associated with early miscarriage

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Study question: Whether the changing peripheral levels of Tim-3/Galectin-9 (Gal-9) and PD-1/PL-1 over 4 weeks after ET in ongoing pregnancies is different from pregnancies destined to miscarry.
Summary answer: A significant and sustained increase of Tim-3 in pNK cells was observed in pregnancies which were ongoing but not in pregnancies which later miscarried.
What is known already: The importance of maternal immune adaptation and tolerance to the implanting embryo, an allograft, has been extensively investigated for decades. Immune checkpoint molecules, like T-cell immunoglobulin mucin-3 (Tim-3) and programed cell death-1 (PD-1), are co-stimulatory receptors negatively regulating immune responses. During pregnancy, Tim-3 and PD-1 are expressed by several immune cells in the decidua and participate in the maternal-fetal immune interactions to mediate maternal immune tolerance through binding to their ligands Gal-9 and progressed death-ligand 1 (PD-L1) produced by trophoblast and immune cells. In addition to the implantation site, Tim-3 and PD-1 expressions in peripheral lymphocytes are modified during pregnancy.
Study design, size, duration: A prospective observational study includes 81 women who achieved ongoing pregnancy and 17 women who suffered from miscarriage after single day-5 blastocyst transfer. All the subjects were recruited from November 2018 to January 2020 in a university teaching hospital.
Participants/materials, setting, methods: Women undergoing single blastocyst transfer after in-vitro-fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment were recruited on the day of ET following informed, written consent. They had serial blood samples taken on the day of ET, and 4, 5, 6 and 7 weeks of gestation for measurement of (1) membranous Tim-3 and PD-1 expression on various peripheral lymphocytes by flow cytometry; and (2) serum concentrations of ligands Gal-9 and PD-L1 by ELISA.
Main results and the role of chance: The comparisons between two groups showed there was no significant difference between the 2 groups in baseline levels among all the parameters measured. In women who achieved ongoing pregnancy, a significant and sustained increase of Tim-3 in either peripheral NK (pNK) subsets was observed at 4-week, 5-week, 6-week and 7-week gestations compared to the baseline (Tim-3+CD56dimNK 39.14±1.51%, 41.14±1.62%, 41.34±1.94%, and 41.69±2.12% vs. 30.27±1.49%; Tim-3+CD56brihgtNK cells, 24.54±1.71%, 25.43±1.54%, 27.26±1.88% and 24.70±1.64% vs. 19.08±1.13%), and the concentration of serum PD-L1 was significantly increased at 6-week and 7-week gestations (48.33±17.78 pg/ml, 52.53±20.60 pg/ml) when compared to the day of blastocyst transfer (41.40±16.01 pg/ml). The expressions of Tim-3 in T, NKT cells and PD-1 in NK, T, NKT cells were not significantly changed across the 5 time points. In women who conceived but later miscarried, all the parameters examined from 4-7 weeks of gestation were not significantly different when compared with the baseline measurement. The only measurement which showed a significant difference between the 2 groups and across all time points after ET was the proportion of Tim-3+CD56dimNK cells which was significantly higher in women who achieved ongoing pregnancies compared with women who destined to miscarry from 4 to 7 weeks of gestation.
Limitations, reasons for caution: It is uncertain if the observation would be different between miscarriage associated with aneuploid embryo or euploid embryo as we had not been able to obtain karyotyping result in most of the miscarriage cases.
Wider implications of the findings: Our preliminary observation suggests that the proportion of Tim-3+pNK cells as early as 4-week gestation could be a potential immuno-bio-marker to predict if a pregnancy is likely to progress normally or result in a miscarriage. Clearly, the finding in this study needs to be confirmed in a larger cohort study
Trial registration number: not applicable.

P-409 Comparison of the incidence of ectopic pregnancies in fresh versus frozen embryo transfers in IVF/ICSI cycles: a meta-analysis of Randomized Controlled Trials (RCT)

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Study question: Is the incidence of ectopic pregnancy (EP) increased in fresh compared with frozen embryo transfer (ET)?
Summary answer: The fresh ET is not associated with an increase of the incidence of EP in comparison with frozen ET.
What is known already: EP represents the first cause of mortality in the first trimester of pregnancy. Assisted reproductive technologies (ART) are associated with an increased EP risk. The reasons of this effect are inadequately explained and may be associated with variables patients-related as tubal diseases and ART-related as the number of embryos transferred, the depth of insertion of the catheter tip during ET and the supraphysiological estradiol levels during ovarian stimulation affecting endometrial receptivity and tubal function. The role of this last factor seems to be corroborated by higher incidence of EP in fresh versus frozen ET, as highlighted by some retrospective studies.
Study design, size, duration: A meta-analysis, based on PubMed, Cochrane CENTRAL, EMBASE, was conducted to estimate and compare the EP rate in fresh versus frozen-thawed ET. Following *PICOS*, inclusion criteria were: *Population*, patients undergoing IVF/ICSI; *Intervention*, fresh ET; *Comparison*, frozen/thawed ET; *Outcome*, EP (primary one), clinical/ongoing pregnancy and live birth rates (secondary ones). *Study design*: RCT.
Participants/materials, setting, methods: Electronic and manual search, conducted from 1990 to 2020, yielded 269 studies. Two researchers reviewed the studies independently, excluding 247 and 16 studies after the first and second screening. The outcome data from the 6 included studies were combined using a Mantel–Haenszel model and applying the random effects models. The dichotomous data results of each study were expressed as risk ratio (RR) with 95% confidence intervals (CI). Heterogeneity was evaluated using the I2 statistic.
Main results and the role of chance: The six studies included in the present review comprise in total 6,675 participants, 3,320 undergoing frozen ET and 3,355 fresh ET (Ferraretti et al., 1999; Chen et al., 2016; Le et al., 2018; Shi et al., 2018; Wei et al., 2019; Stormlund et al., 2020).

Preliminary analyses excluded interaction between covariates, defining intervention/control groups and outcome. In particular, the incidence of tubal infertility was comparable between the two groups. Risk ratio of EP ranged from 0.03 (Ferraretti et al., 1999) to 2.77 (Shi et al., 2018). The level of heterogeneity (I2) between studies was 42% and it was considered as moderate. EP incidence resulted not significantly different in fresh ET [56/1,703 pregnancies] compared with frozen ET [44/1,799 pregnancies] (RR=0.450, 95% CI 0.13-1.81, p=0.29; I2=42%).

Analysis of the secondary outcomes was conducted on 5 studies; results showed that clinical pregnancy rate was not significantly different between fresh (0.52) and frozen ET (0.56) (RR=1.11; 95% CI= 0.93-1.34); at the same manner, the ongoing pregnancy rate (RR=1.11; 95%CI=0.91-1.37; 0.46 vs 0.49 for fresh and frozen, respectively) and the live birth rate (RR=0.93; 95%CI=0.60-1.44; 0.47 vs 0.49 for fresh and frozen, respectively) resulted not significantly different between the two groups.
Limitations, reasons for caution: The EP incidence was not the primary outcome of the included RCTs. This could have determined a sample size not calibrated for the analysis of the primary outcome of the present meta-analysis.
Wider implications of the findings: This meta-analysis indicates that EP incidence is similar between fresh and frozen ET. The possible role on EP risk of the supraphysiological estradiol levels during ovarian stimulation should be reconsidered. Based on these results, the choice of a frozen ET should not derive by the presumed reduction of EP risk.
Trial registration number: Not applicable

P-410 Comparison of the incidence of miscarriage rates in frozen versus fresh embryo transfers in IVF/ICSI cycles: a meta-analysis of Randomized Controlled Trials (RCT)

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Study question: Is the incidence of miscarriage rates increased in frozen embryo transfer (ET) compared with fresh ET?
Summary answer: The frozen ET is not associated with an increased risk of miscarriage in comparison with fresh ET.
What is known already: Nowadays, the freeze-all policy has gained great popularity because it minimizes OHSS and it seems to improve the reproductive outcomes. This increase was possible thanks to progress in vitrification procedures. Nevertheless, some recent evidence suggests more caution on the wide use of elective frozen ET in a general IVF population for its possible effect on some pregnancy-related complications.

Endometrial preparation schemes for frozen ET include natural cycles or, more frequently, artificial cycles in which the endometrium is prepared with exogenous steroids. In this last case, the corpus luteum is absent, but its demise could be correlated with some pregnancy-related complications.
Study design, size, duration: A meta-analysis, based on PubMed, Cochrane CENTRAL, EMBASE, was conducted to estimate and compare the miscarriage rate (MR) in frozen versus fresh ET. Following *PICOS*, inclusion criteria were: *Population*, patients undergoing IVF/ICSI; *Intervention*, frozen ET; *Comparison*, fresh ET; *Outcome*, MR (primary one); clinical pregnancy rate (CPR), ongoing pregnancy rate (OPR) and live birth rates (LBR) (secondary ones). *Study design*: RCT.
Participants/materials, setting, methods: Electronic and manual search, conducted from 1990 to 2020, yielded 269 studies. Two researchers reviewed the studies independently, excluding 247 and 14 studies after the first and second screening. The outcome data from the 8 included studies were combined using a Mantel–Haenszel model and applying the random effects models. The dichotomous data results of each study were expressed as risk ratio (RR) with 95% confidence intervals (CI). Heterogeneity was evaluated using the I2 statistic.
Main results and the role of chance: The eight studies comprise 6,934 participants, 3,450 undergoing frozen ET and 3,484 fresh ET (Ferraretti et al., 1999; Shapiro et al., 2011a; Shapiro et al.; 2011b; Chen et al., 2016; Shi et al., 2018; Le et al., 2018; Wei et al., 2019; Stormlund et al., 2020).

Preliminary analyses excluded interaction between covariates. RR of miscarriage ranged from 0.29 (Chen et al., 2016) to 3.24 (Ferraretti et al., 1999). The level of heterogeneity (I2) was 48% and it was considered as moderate. MR resulted not significantly different in frozen [279/1,601 pregnancies] compared with fresh ET [308/1,458 pregnancies] (RR=0.727, 95%CI=0.43-1.25, p=0.25). By analysing the 5 trials in which the endometrium was prepared with steroids (Ferraretti et al., 1999; Shapiro et al., 2011a; Shapiro et al., 2011b; Chen et al., 2016; Le et al., 2018), MR resulted not significantly different in frozen [126/720] versus fresh ET [160/682] (RR=0.605, 95%CI 0.22-1.66; p=0.33; I2=43%).

For the secondary outcomes, CPR (RR=1.14; 95%CI=0.97-1.35; 0.56 vs 0.52 for frozen and fresh ET, respectively), OPR (RR=1.12; 95%CI=0.95-1.33; 0.49 vs 0.46 for frozen and fresh ET, respectively) and LBR (RR=0.93; 95%CI=0.60-1.44; 0.49 vs 0.47 for frozen and fresh ET, respectively) were not significantly different between the two groups.
Limitations, reasons for caution: The MR was not the primary outcome of the included RCTs. This could have determined a sample size not calibrated for the analysis of the primary outcome of the present meta-analysis.
Wider implications of the findings: This meta-analysis indicates that MR was similar between frozen and fresh ET.

The endometrial preparation, in artificial cycles without corpus luteum, does not seem to influence the good course of pregnancy. Based on these results, the choice of a fresh ET should not derive by the presumed reduction of MR.
Trial registration number: Not applicable

P-411 Does subcutaneous progesterone (SC-P) administration eliminate the necessity of serum progesterone level monitoring in frozen embryo transfer (FET) cycles?

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Study question: Does SC-P provide similar ongoing pregnancy rates (OPRs) as intramuscular progesterone(IM-P) in hormone replacement therapy

(HRT)-FET cycles and do serum progesterone (P) levels on FET day effect on pregnancy outcome? Summary answer: SC-P administration had similar OPR compared to IM-P in HRT-FET cycles. In SC-P group embryo transfer(ET) day P found to be insignificant factor for outcome.

What is known already: Different P routes can be used in HRT-FET cycles such as vaginal P, IM-P and recently SC-P. Only retrospective studies evaluated the comparison of SC-P with other routes in HRT-FET cycles. Here, we assessed prospectively whether SC-P is effective for HRT-FET cycles. Previous studies reported that serum P levels on ET day after vaginal P administration clinical outcomes were closely correlated. The correlation between serum P after IM-P administration and clinical outcomes were conflicting. In addition, there is lack of data on the serum P levels after SC-P administration. Serum P levels on ET day were evaluated in this study.

Study design, size, duration: This prospective cohort study was performed between July 1-October 31 2020, enrolled 224 patients scheduled for HRT-FET cycles with SC-P(25 mg twice daily) or IM-P(50 mg once daily). The route of P was decided according to the patient's eligibility to hospital. First FET cycle was included after freeze-all cycles for each patients. Female age>35, PGT-A cycles, cleavage ET, >1 ET, patients with uterine pathology and hydrosalpinx, FET with surplus embryos, endometrial thickness<7mm were excluded.

Participants/materials, setting, methods: Female age ≤ 35 years old with a triple-layer endometrium >7 mm underwent transfer of single blastocysts after the first ET after freeze-all cycles. The indications for freeze-all were ovarian hyperstimuation syndrome and trigger day P level>1.5 ng/ml. 224 patients were eligible for study; 133 in SC-P group and 91 in IM-P group.The primary endpoint was the ongoing pregnancy rate (OPR) beyond pregnancy week 12.

Main results and the role of chance: The demographic, cycle, embryologic characteristics were similar between groups. The median circulating P levels on the day of ET were 19.92(15.195-27.255)ng/ ml and 21(16.48-28)ng/ml in the SC-P and IM-P groups,(p=0.786). The clinical pregnancy rates [86/133(64.7%) vs 57/91(62.6%);p=0.757], miscarriage rates [21/86(24.4%) vs 10/57(17.5%) ;p=0.329], and OPR [65/133 (48.9%) vs 47/91(51.6%); p=0.683] were comparable between the SC-P and IM-P. Binary logistic regression was performed for ongoing pregnancy as the dependent factor blastocyst morphology was found to be the only significant independent prognostic factor (p = 0.006), whereas the route of P was insignificant. In the SC-P and IM-P groups, the effect of ET day P levels were divided into quartiles(Q) to evaluate the effect on ongoing pregnancy. In SC-P group OPR were similar in four Q [Q1:33.3%(11/33),Q2:50%(17/34),Q3:60.6%(20/33),Q4:51.5%(17/33) (p=0.1)].For IM-P group; Q1 had a significantly reduced OPR than Q2, Q3, Q4. [26.1%(6/23),65.2%(15/23),54.5%(12/22) and 60.9%(14/23), p=0.031]. Logistic regression analysis for OP was performed separately in SC-P group and IM-P group. Although in SC-P group, ET day P levels was not found to be a significant factor, in IM-P ET day P level was found to be an independent factor for OP in IM-P group (Q1vs Q2+Q3+Q4; OR: 8,178 95% CI: [1.387-48.223] p:0.02).

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Limitations, reasons for caution: Although this study has the advantage of being prospective and in a homogenous study population, randomized controlled trials are warranted to evaluate the effectiveness of SC-P to other routes of P. Extrapolation to unselected populations of this study is needed.

Wider implications of the findings: Assignment of threshold of serum P on the day of ET for HRT-FET cycles to optimize outcomes is critical for every route of P. Regarding these results, individual luteal phase for HRT-FET cycles can improve IVF outcome.

Trial registration number: None

P-412 Low serum hCG levels adjusted for the hCG trigger dose in fresh cycles may be associated with a poor clinical pregnancy rate of FET cycles

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Study question: Is there any association between serum human chorionic gonadotropin (hCG) levels after trigger at previous fresh cycles and pregnancy outcomes of frozen-thawed embryo transfer (FET) cycles?

Summary answer: Low adjusted serum hCG level after hCG trigger at fresh cycles is negatively associated with clinical pregnancy rates (CPR) of hormone replacement treatment-FET (HRT-FET). What is known already: Literature showed that low serum hCG levels after the same dose of hCG trigger was associated with reduced pregnancy outcomes of the fresh cycles. However, the relationship between hCG levels after trigger at fresh cycles and pregnancy outcomes of FET cycles remains unknown.

Study design, size, duration: This matched retrospective study was conducted at a Reproductive Medicine Center between 2016 and 2018. Subjects performing HRT-FET cycles, whose previous fresh cycles used a bolus of hCG alone or a bolus of GnRHa combined with hCG for trigger were included. A total of 186 HRT-FET cycles with complete data was included for the final analysis.

Participants/materials, setting, methods: The study population was grouped into women with intramuscular injection of hCG prior to secretory transformation (hCG group, n = 93) and a comparison group (control group, n = 93) of women without hCG addition matched for patients' age and duration of infertility. At the previous fresh cycles, serum hCG levels were measured 12 hours later after hCG trigger (defined as the "hCG+12 h" timepoint), and were adjusted for doses (defined as adjusted hCG levels). Main results and the role of chance: For patients achieving clinical pregnancy, the adjusted hCG level significantly increased ($P<0.05$). Meanwhile, the ROC curve also showed a significantly predictive value of adjusted serum hCG levels at the "hCG+12 h" timepoint for CPR in HRT- FET cycles (AUC=0.626, 95%CI: 0.512-0.740) and the optimal hCG threshold proposed by ROC for CPR was 46.31 mIU/mL with sensitivity of 71.4% and specificity of 56.9%. For all patients, the CPR in hCG group was significantly higher than that in control group (61.3% vs. 44.1%). Furthermore, all cycles were then divided into four groups based on the injection of hCG prior to secretory transformation in HRT-FET cycles and this cut-off value of hCG levels at the "hCG+12 h" timepoint. For patients with adjusted hCG levels ≤46.31 mIU/mL, the CPR was significantly improved in hCG group compared with control group (61.1% vs. 29.3%). But for patients with adjusted hCG levels >46.31 mIU/mL, no statistically significant difference was observed between the hCG and control group (61.4% vs. 55.8%).

Limitations, reasons for caution: Although the results achieved statistically significant, the sample size was relatively small, which limits our ability to draw a definitive conclusion. The reason of the small sample size may be that to reduce the risk of OHSS, doctors would give preference to trigger with GnRH agonist in our center.

Wider implications of the findings: Adjusted serum hCG levels might represent a potential factor to guide adequate support in the subsequent HRT-FET cycles. Meanwhile, for patients with low adjusted serum hCG levels, intramuscular hCG injection prior to secretory transformation may be a good compensation way to rescue pregnancy impair in the subsequent HRT-FET cycles.

Trial registration number: N/A.

P-413 The combination of endometrial injury and Freeze-All strategy in women with repeated implantation failures

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Study question: Can the combination of hysteroscopic endometrial injury (EI) and freeze-all strategy improve pregnancy parameters, mainly live birth, in women with repeated implantation failures (RIF)?

Summary answer: The combination of Endometrial Injury and freeze-all strategy has no significant effect on live birth, clinical and miscarriage rates in RIF patients undergoing ART.

What is known already: A variety of strategies and approaches for RIF patients undergoing ART have been used and proposed. Currently there is insufficient evidence in the literature concerning the effect of either EI or freeze-all strategy in IVF cycles and very limited on the combination of these two approaches in RIF patients.

Study design, size, duration: This is a two-center two-arm cohort study conducted at both University and Private Assisted Reproductive Units in Greece, encompassing 60 cycles with vitrification as the cryopreservation method from 60 participants during the last three years.
Participants/materials, setting, methods: The study group comprised of 30 patients with RIF and underwent a hysteroscopic endometrial injury in the menstrual cycle prior that to the embryo transfer. The control group comprised of patients with RIF and underwent a standard cycle with no adjuvant treatment. Our primary analysis was performed to provide a direct comparison between groups. Logistic and Poisson Regression models were further employed to examine possible confounding effects.
Main results and the role of chance: Live birth did not differ between groups (p=0.0953); similarly, clinical pregnancy and miscarriage rates were comparable among them (p=0.3472 and p=0.2542, respectively). The number of retrieved oocytes was the only significant confounder for biochemical pregnancy (p=0.0481, 95% CI: (0.0014, 0.3223)].
Limitations, reasons for caution: Limitations of the study include the lack of randomization that is linked with known and unknown biases and the small cohort size.
Wider implications of the findings: The combination of both endometrial injury and freeze-all strategy does not appear to improve pregnancy rates, including live birth, in patients with RIF undergoing ART.

The number of retrieved oocytes was the only significant confounder for biochemical pregnancy.
Trial registration number: NCT04597463

P-414 HLA-G expression as a new prognostic marker of successful implantation

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Study question: Is the low HLA-G expression a determinant of early reproductive loss?
Summary answer: Low expression of HLA-G is associated with pregnancy complications and can be one of the reasons of spontaneous abortion (such as RPL).
What is known already: The dysregulated maternal immune responses to invading embryos may play role in RIF, RPL, and second- and third-trimester obstetrical conditions. HLA-G is a molecule that was first known to confer protection to the fetus from destruction by the immune system of its mother, thus critically contributing to fetal–maternal tolerance due to inducing displacement of pro-inflammatory to Th1 cell-mediated response of Th2, has a positive influence on the process of implantation. HLA-G is mainly restricted to the fetal–maternal interface on the extravillous cytotrophoblast, to placenta, amnion.
Study design, size, duration: It was a prospective complex cohort study from 2016-2020 years with pathomorphological investigations. The purpose of this study is to investigate the HLA-G and KIR2DL4 expression in chorionic villous among 3 groups (study included 118 cases of abortion material): group 1 – 36 cases after missed abortion with normal karyotype, group 2 – 36 cases after missed abortion with polyploidy and group 3 – 46 cases after induced abortion group (normal pregnancy).
Participants/materials, setting, methods: Criteria of inclusion: abortive material from 3 groups of women with missed or after induced abortion; 6-12 weeks, singleton pregnancy, cytogenetic of chorionic villous was obligatorily - normal fetal karyotype and polyploidy of fetus. Pathomorophological investigation included H&E stain, IHC and confocal laser scanning microscopy. Immunohistochemical examination included quantitative and qualitative assessment of the expression of Anti-HLA-G (mouse monoclonal) in an extra villous trophoblast and Anti-KIR2DL1+KIR2DL3 + KIR2DL4 + KIR2DS4 (rabbit polyclonal) in chorionic villi.
Main results and the role of chance: The immunohistochemical study showed homogenous distribution HLA-G expression in extravillous trophoblast cells (EVT) and KIR2DL4 expression in chorion villous both in missed abortion groups and in induced abortion group. HLA-G expression average relative area in 1 and 2 groups was not statistically different (in 1 group with normal karyotype 33,9±3,5 and in 2 group 38,6±2,8). But the expression of HLA-G in 3 group was strictly higher (55,6 ±2,4). The average relative area of KIR2DL4 receptor wasn't statistically different among 3 groups. However, the histological picture both missed abortion groups (for the genetic\immunological reasons for rejection) is the only one - this is a missed abortion of an early terms of gestation. In a histological study of missed abortion, as our study shows, the histological picture is similar in 1 and 2 groups. Thus, in 1 group with a normal karyotype of the fetus (before conducting the chorion cytogenetic study in the genetics laboratory) in 59.2% the histological examination determined a picture of an impaired early pregnancy with signs of trophoblast chromosomal pathology. Thus, without a cytogenetic study of the chorion, it is impossible to clearly determine whether the chromosomal pathology of the fetus is the cause of missed abortion.
Limitations, reasons for caution: There is no limitations, reasons for caution.
Wider implications of the findings: Thus, HLA-G molecule has a leading role in the onset and successful prolongation of pregnancy, implantation, placentation and fetal development.
Trial registration number: 98-2019

P-415 Clinical and In Vitro Fertilization laboratory parameters that contribute to clinical miscarriage after single euploid embryo transfer

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Study question: To investigate which factors, excluding embryo aneuploidies, are associated with miscarriage in patients who have undergone a single euploid blastocyst transfer.
Summary answer: Miscarriage was related to the body mass index (BMI), the type of cycle and the thickness of the endometrium.
What is known already: Preimplantation genetic testing for aneuploidies (PGT-A) is widely used in-vitro fertilization (IVF) to select euploid embryos. Several studies have shown that embryo aneuploidy is the main contributing factor for IVF failure, reinforcing the relevance of PGT-A as a method to select chromosomally normal embryos.

A recent meta-analysis confirmed that patients undergoing PGT-A have a lower miscarriage rate than women that conceived naturally (9% vs 28%, respectively).

Even though most of the studies show that PGT-A significantly reduces miscarriage rate, still some women do lose their pregnancies. We investigated which other reasons may be related to this early pregnancy loss.
Study design, size, duration: Retrospective, observational, and multicenter study of 6910 patients undergoing single euploid blastocyst transfer after PGT-A from January 2017 to December 2019 in our institution. Several laboratory and clinic variables were analyzed to study the effect of these variables on the miscarriage rate
Participants/materials, setting, methods: Indications for PGT-A were advanced maternal age, implantation failure, recurrent pregnancy loss and male factor. Embryos were cultured 5% O2 concentration and 6.5% CO2 concentration. Trophectoderm biopsy was performed on day 5/6 of development and analyzed through Next Generation Sequencing (NGS); embryos were vitrified until transfer was performed. Single euploid embryo transfer was performed in all cases.

We performed a multivariate regression analysis to compare the different variables and search for there are significant differences.
Main results and the role of chance: We studied a total of 6910 patients undergoing PGT-A to describe which factors, excluding embryo aneuploidies, were correlated with miscarriage in patients who underwent single thawed euploid embryo transfer.

When considering embryo morphology (embryo grading, quality of inner cell and quality of trophectoderm), we did not find differences in miscarriage rate among groups (high quality= 15.9%; normal quality= 14.3%; low quality= 15.0%; poor quality= 14.8%) p = 0.833.

BMI was significantly associated with miscarriage rate (odds ratio [OD] 1.04; 95% confidence interval [CI], 1.012-1-076 p=0.006) and miscarriage rate.

We observed a weak association between endometrial thickness and miscarriage rate ([OD] 0.65; 95%, 0.528-0.778 p=0.04) and also between type of endometrial preparation (natural cycle or hormone replacement cycle) ([OD] 0.77; 95%, 0.528-0.778) p=0.04.

Body mass index, according to our findings, was the main variable correlated with miscarriage rate. We did not find any association with the other variables studied (biopsy day, maternal age, male age, duration infertility, cycle length, previous miscarriage, previous live birth, previous cycles IVF, endometrial pattern and diagnosis).

Limitations, reasons for caution: The retrospective study design limits the generalization of our results but offers a good insight to be validated in prospective trials.

Wider implications of the findings: According to our findings, BMI, endometrial thickness the day of the embryo transfer, and the type of endometrial preparation should be considered when transferring an euploid blastocyst.

Trial registration number: NO APLICA

P-416 Radiotherapy inflicts long-term damage upon the uterus, causing uterine artery endothelial dysfunction and pregnancy loss in mice

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Study question: Does the uterus sustain direct and long-term damage following radiotherapy, independent of ovarian damage?

Summary answer: Radiotherapy causes direct and long-term uterine damage. Ovariectomised and hormonally supplemented mice experience uterine artery endothelial dysfunction and pregnancy loss following transfer of healthy blastocysts.

What is known already: The detrimental off-target impacts of cancer therapies on the ovary are well established, with some fertility preservation techniques available to ensure patients maintain their fertility following gonadotoxic treatment. Low doses of radiotherapy kill the majority of primordial follicle oocytes in the ovary, reducing the ovarian reserve and fertile lifespan. Patients who have received radiotherapy experience higher rates of pregnancy complications including preterm birth, low birth weight, and stillbirth. However, no studies have investigated if radiation inflicts direct, fertility compromising damage to the uterus.

Study design, size, duration: Adolescent female mice were untreated or exposed to whole body y-irradiation (7Gy), then ovariectomised. Immediate damage was assessed up to 24 hours post-irradiation (n=4/group). Four weeks following treatment, mice were hormone primed to induce endometrial receptivity (n=7/group), artificial decidualisation (n=7-8/group), or receive embryo transfers from healthy, unexposed donor mice to assess embryo implantation (n=11-13/group), and mid-gestation development (n=8-10/group).

Participants/materials, setting, methods: Four week old C57BL6/CBA (F1) female mice were used for this study. Immunofluorescence and in situ hybridisation were utilised to localise markers of immediate DNA damage and cell death following irradiation, and markers of receptivity in hormone primed uteri. Measurements of uterine artery blood flow were recorded using Doppler ultrasonography, and indices of pulsatility and resistance calculated. Uterine artery wire myography was performed to assess competency of endothelial and smooth muscle compartments following irradiation.

Main results and the role of chance: Within 24 hours of irradiation, DNA damage (γH2AX) and apoptosis (Puma/TUNEL) were elevated in uteri, compared to control. Irradiated mice that received embryo transfers from untreated donors had similar numbers of implantation sites 3-days post-transfer versus controls, however uteri were pale and atrophic suggesting impaired vascularisation. By 10-days post-transfer, implantation sites in irradiated mice were resorbing ($p<0.001$), although uterine artery Doppler ultrasound measurements demonstrated no change in pulsatility or resistance indices. When the brain was shielded from irradiation to protect the hypothalamic-pituitary-gonadal axis, resorption still occurred ($p<0.001$), suggesting direct uterine damage is the likely cause of pregnancy loss. To investigate uterine damage in the absence of an embryo, endometrial receptivity was induced artificially. Uteri from irradiated animals were lighter compared to control ($p<0.05$), however localisation of receptivity markers (E-cadherin, Mucin1, Ki67) was normal. When decidualisation was artificially induced irradiated uteri were also lighter ($p<0.01$) indicating impaired decidualisation and reduced capacity to adapt to pregnancy. Wire myography performed on uterine arteries demonstrated endothelial dysfunction in irradiated mice ($p<0.0001$).

Limitations, reasons for caution: Here, only a single age and dose of radiotherapy exposure are modelled. Patients of all ages can receive many doses of radiotherapy in combination with various chemotherapies. Our highly manipulable model enables any treatment variation to be modelled and the effect on the uterus and pregnancy examined.

Wider implications of the findings: Reproductive aged cancer patients need to be appropriately counselled regarding the risks to their long-term fertility following treatment. Until now, potential permanent impacts to the uterus following cancer treatment have not been considered. It is clear radiotherapy can impose long-term damage to the uterus and influence pregnancy success and fertility.

Trial registration number: NA

P-417 A comparison of baseline and sequential changes of extended cytokine profile during implantation window between women who did and did not conceive after embryo transfer

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Study question: To compare the changing peripheral levels of inflammation-related cytokine profile during a 9-day period after blastocyst transfer between women who did and did not conceive.

Summary answer: Successful implantation is associated with transient increase in serum pro-inflammatory cytokine profile followed by a switch to anti-inflammatory cytokine profile prior to confirmation of pregnancy.What is known already: Immunomodulation is thought to be important for the prevention of rejection of the implanting semi-allograft embryo and successful establishment of pregnancy. A successful pregnancy is characterized by a dominance of anti-inflammatory cytokine profile in the peripheral blood in the first and second trimesters of pregnancy. It is achieved by a complex interplay between various immune cells and cytokines at the fetal-maternal interface, among which the key-players are interleukine-10 (IL-10) and transforming growth factor- 1 (TGF- 1). The circulating inflammatory response in the first few days after embryo transfer to the pathophysiology of implantation failure remains unclear. Study design, size, duration: This prospective observational and longitudinal study on 47 women with infertility was performed in an in vitro fertilization unit from December 2018 to August 2019. The amounts of a range of cytokines was measured on serial blood samples obtained during a 9-day period after blastocyst transfer.

Participants/materials, setting, methods: Serial blood samples were obtained on the day of embryo transfer, and 3, 6, and 9 days afterward for measurement of serum interferon gamma (IFN-), tumor necrosis factor alpha, interleukin (IL)-2, IL-4, IL-10, IL-12, IL-13, IL-17, IL-18, and IL-22 using cytometric bead arrays; transforming growth factor beta 1 (TGF- 1) was measured using commercial enzyme-linked immunosorbent assay kits.

Main results and the role of chance: The cytokine profile was similar between the women who conceived and those who did not on the day of blastocyst transfer. In women who conceived, IFN- and IL-17 (pro-inflammatory cytokines) exhibited a transient and significant increase on day 3 after blastocyst transfer, which decreased to the baseline levels by day 6. Meanwhile, IL-10 (anti-inflammatory cytokine) was increased significantly on days 6 and 9, and TGF- 1 (anti-inflammatory cytokine) was increased significantly on day 9 after blastocyst transfer. In women who did not conceive, there was a more

pronounced increase in IFN- and IL-17 (pro-inflammatory cytokines) on day 3, which was sustained on days 6 and 9 without a switch to an anti-inflammatory cytokine profile. Among women who conceived after blastocyst embryo transfer, there was a transient and modest increase in serum pro-inflammatory cytokine profile (IFN- and IL-17) 3 days after blastocyst transfer, which was followed by a switch to anti-inflammatory cytokine profile (increase IL-10 and TGF- 1) by 6 days after blastocyst transfer and the latter increase was sustained 9 days after blastocyst transfer, when pregnancy was confirmed.

Limitations, reasons for caution: This is an observational study on peripheral blood cytokine levels, so it is not possible to draw conclusions if the implantation failure is due primarily to failure of the embryo to elicit a trigger for the switch or failure of maternal response to a normal trigger released by the embryo.

Wider implications of the findings: The characteristic change in peripheral cytokine profile during successful implantation, well before confirmation of pregnancy, may provide an opportunity to develop serum biomarkers to monitor implantation and to understand the mechanism of its failure, especially in women who experience recurrent implantation failure after IVF.

Trial registration number: not applicable

P-418 Different immunoregulatory components at the decidua basalis of oocyte donation pregnancies

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Study question: Is the number of regulatory T-cells (Tregs) and immunoregulatory cytokines in the decidua basalis of oocyte donation (OD) pregnancies different compared to naturally conceived pregnancies?

Summary answer: This study suggests that the immunoregulation at the fetal-maternal interface in OD pregnancies with a higher amount of fetal-maternal HLA mismatches appears to be altered.

What is known already: Tregs and related immunoregulatory cytokines, such as interleukins, transforming growth factor-β, and galectin-1, play a key role in maintaining tolerance at the decidua basalis in human pregnancy. Previous studies observed decreased numbers of decidual Tregs in miscarriage and preeclamptic pregnancies. These complications occur in higher frequencies in OD pregnancies, which are characterized by more fetal-maternal human leukocyte antigen (HLA) mismatches compared with naturally conceived (NC) and non-donor in vitro fertilization (IVF) pregnancies, since the fetus obtains paternal and donor-derived HLA genes. Consequently, the maternal immune system has to cope with greater immunogenetic dissimilarity. Involved immunoregulatory mechanisms however remain poorly understood. Study design, size, duration: This case-control study included 27 OD, 11 IVF, and 16 NC placentas of uncomplicated pregnancies, which were collected after delivery at 37-42 weeks of gestation between 2005 and 2013. Clinical data, maternal peripheral blood and umbilical cord blood were collected.

Participants/materials, setting, methods: Decidua basalis was dissected from the placentas, and processed to formalin-fixed, paraffin-embedded slices (4 μm). Immunohistochemical staining for FOXP3, interleukin 10, interleukin 6, galectin-1, transforming growth factor- , and Flt-1 was performed. Semi-quantitative (FOXP3+ Tregs) and computerized analysis (cytokines), using Image-J software, were executed. Maternal peripheral blood and fetal umbilical cord blood were typed for HLA class I and II, using the Sequence Specific Oligonucleotides PCR technique, to calculate the number of fetal-maternal HLA mismatches.

Main results and the role of chance: All the deciduae basalis of OD, IVF and NC pregnancies showed FOXP3+ Tregs. No significant differences were found when comparing the three groups for the mean number of FOXP3+ Tregs. However, when the amount of fetal-maternal HLA mismatches was related to the percentage of FOXP3+ Tregs, the Tregs were significantly higher in pregnancies with 4-6 HLA class I mismatches (n = 16), than in those with 0-3 HLA class I mismatches (n = 38; p = 0.029). Furthermore, OD pregnancies express less interleukin 10, interleukin 6, galectin-1 and Flt-1 in the decidua basalis compared to NC pregnancies. Moreover, the amount of interleukin 10 was significantly lower with 3-4 fetal-maternal HLA class II mismatches (p = 0.032).

Limitations, reasons for caution: This study is limited by a small sample size. Moreover, only term placentas were collected. It would be worthwhile investigating immunological alterations in the decidua throughout the whole gestation, since maternal adaptation of the fetal allograft could be more prominent early in pregnancy.

Wider implications of the findings: Unravelling the mechanisms of immunomodulation during OD pregnancy, reflected by a high level of fetal-maternal dissimilarity, could help to reach the ultimate goal in transplantation; the induction of donor-specific tolerance. In addition, it might help to understand the development of complications in OD pregnancy.

Trial registration number: Not applicable

P-419 On prognosis after unexplained recurrent pregnancy losses (RPL); a systematic review and external validation of clinical prediction models

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Study question: Which models that predict pregnancy outcome in couples with unexplained RPL exist and what is the performance of the most used model?

Summary answer: We identified seven prediction models; none followed the recommended prediction model development steps. Moreover, the most used model showed poor predictive performance.

What is known already: RPL remains unexplained in 50-75% of couples For these couples, there is no effective treatment option and clinical management rests on supportive care. Essential part of supportive care consists of counselling on the prognosis of subsequent pregnancies. Indeed, multiple prediction models exist, however the quality and validity of these models varies. In addition, the prediction model developed by Brigham et al is the most widely used model, but has never been externally validated.

Study design, size, duration: We performed a systematic review to identify prediction models for pregnancy outcome after unexplained RPL. In addition we performed an external validation of the Brigham model in a retrospective cohort, consisting of 668 couples with unexplained RPL that visited our RPL clinic between 2004 and 2019.

Participants/materials, setting, methods: A systematic search was performed in December 2020 in Pubmed, Embase, Web of Science and Cochrane library to identify relevant studies. Eligible studies were selected and assessed according to the TRIPOD) guidelines, covering topics on model performance and validation statement. The performance of predicting live birth in the Brigham model was evaluated through calibration and discrimination, in which the observed pregnancy rates were compared to the predicted pregnancy rates.

Main results and the role of chance: Seven models were compared and assessed according to the TRIPOD statement. This resulted in two studies of low, three of moderate and two of above average reporting quality. These studies did not follow the recommended steps for model development and did not calculate a sample size. Furthermore, the predictive performance of neither of these models was internally- or externally validated.

We performed an external validation of Brigham model. Calibration showed overestimation of the model and too extreme predictions, with a negative calibration intercept of -0.52 (CI 95% -0.68 – -0.36), with a calibration slope of 0.39 (CI 95% 0.07 – 0.71). The discriminative ability of the model was very low with a concordance statistic of 0.55 (CI 95% 0.50 – 0.59).

Limitations, reasons for caution: None of the studies are specifically named prediction models, therefore models may have been missed in the selection process. The external validation cohort used a retrospective design, in which only the first pregnancy after intake was registered. Follow-up time was not limited, which is important in counselling unexplained RPL couples.

Wider implications of the findings: Currently, there are no suitable models that predict on pregnancy outcome after RPL. Moreover, we are in need of a model with several variables such that prognosis is individualized, and factors from both the female as the male to enable a couple specific prognosis.

Trial registration number: not applicable

P-420 uncomplicated oocyte donation pregnancies display elevated CD163 positive type 2 macrophage load in the decidua, which is associated with fetal-maternal HLA class II mismatches

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Study question: Do quantity and composition of decidual macrophages differ between uncomplicated oocyte donation (OD) pregnancies and non-OD in vitro fertilization (IVF) pregnancies?
Summary answer: OD placentas show higher decidual CD163 positive fraction within the total macrophage population compared to non-OD IVF placentas.
What is known already: The embryo of an OD pregnancy is completely allogeneic to the mother, which may lead to a bigger challenge for the maternal immune system to tolerize the fetus compared to autologous pregnancies. Placental macrophages may be essential in maintaining a healthy pregnancy. Macrophages can be classified into different categories based on phenotype and characteristics, in which type 2 macrophages are thought to exhibit immune suppressive activity.
Study design, size, duration: This retrospective case-control study included patients who delivered in the Leiden University Medical Center between January 1st 2006 and July 1st 2016. A total of 42 pregnancies were enrolled in this study, conceived by uncomplicated singleton OD pregnancies (n=25) or non-OD IVF pregnancies (n=17). Medical records were reviewed and clinical data were collected. Placental tissue samples were collected for immunohistochemical staining and blood samples were collected for HLA typing.
Participants/materials, setting, methods: Placentas were collected and immunohistochemically stained for CD14 (pan-macrophage marker) and CD163 (type 2 macrophage marker). The extent of staining was quantitated by digital image analysis software. To assess mismatching, maternal and fetal DNA was typed for HLA-A, -B, C, -DRB1, and -DQB1.
Main results and the role of chance: A significantly lower percentage of CD14 positive staining was observed in the decidua basalis of OD pregnancies compared to non-OD IVF pregnancies (p=0.030). Consequently, the CD163/CD14 ratio in OD group was higher than in non-OD IVF group (p=0.243). In the parietalis, OD pregnancies demonstrated a significantly higher percentage of CD163+ staining (p=0.040) and a significantly higher CD163/CD14 ratio (p=0.032) compared to non-OD IVF group. The reproducibility of this quantitative analysis was found to be high. OD group was separated into a syngeneic group (number of mismatches lower than half of the antigens per HLA locus) and an allogeneic group (number of mismatches higher than half of the antigens per HLA locus). Significant differences of CD163+ and CD163/CD14 ratio were found in the decidua parietalis when comparing the HLA-classII-allogeneic OD group with the non-OD IVF group (p=0.047). This difference was not found for the HLA-class-II-syngeneic OD group.
Limitations, reasons for caution: Our study only focused on decidua basalis and parietalis, no other locations in the placentas. Larger sample size might be needed to verify the association between macrophages and HLA mismatches.
Wider implications of the findings: To our knowledge, this study is the first to quantify a higher CD163 positive M2 macrophages load within the total decidual macrophages of uncomplicated OD pregnancy compared to non-OD IVF pregnancies.
Trial registration number: not applicable

P-421 Down-expression of glycolytic pathway-related protein A1 is associated with the pathogenesis of recurrent pregnancy loss

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Study question: How does glycolytic pathway-related protein A1 (GPRPA1) relate to the pathogenesis of recurrent pregnancy loss (RPL)?
Summary answer: GPRPA1 was found as a substrate of ITI-H4 to modulate the inflammatory response and was down-expressed in the sera of RPL patients.
What is known already: Thus far, the pathogenesis of RPL was not fully understood. In a previous study, the short isoform ITI-H4 cleaved by kallikrein B1 was detected in the sera of RPL patients and would be an important inflammatory factor for RPL by increasing pro-inflammatory cytokines. GPRPA1, a new binding partner of ITI-H4, was known to relate with pre-eclampsia and human decidualization by regulating angiogenesis and glycolysis. Also, GPRPA1 affects placental cell motility and cancer cell proliferation.
Study design, size, duration: Through immunoprecipitation (IP) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) analyses, we found new binding partners of ITI-H4. Of these, GPRPA1 was selected, and direct binding between GPRPA1 and ITI-H4 was confirmed by IP and GST pull-down assay. Differential expression of GPRPA1 in sera and cellular functions of GPRPA1 in the placental cell line were investigated by molecular and cellular analyses.
Participants/materials, setting, methods: The Flag-tagged full-length ITI-H4 and the short isoform ITI-H4 were transfected into HEK293T cells and IP has proceeded with the Flag antibody. Spots showing differential expression were analyzed by MALDI-TOF/MS analysis and peptide sequence alignment was performed. The binding between GPRPA1 and ITI-H4 was confirmed using IP and GST pull-down assay. The effects of GPRPA1 on cellular functions in the placental cell were investigated by CCK-8 assay, invasion assay, and colony-forming assay.
Main results and the role of chance: Through IP, MALDI-TOF/MS analysis, and peptide sequence alignment, we found new substrates of ITI-H4 related to glycolysis, T cell activation, and production of thyroid hormones. Of these, we selected GPRPA1 which is secreted in the serum to utilize a serum biomarker of RPL. GPRPA1 directly binds to the full-length ITI-H4 and also binds to the short isoform ITI-H4 shown by IP and GST pull-down assay. Besides, GPRPA1 as a protein kinase increases serine phosphorylation of ITI-H4 and inhibits the cleavage by KLKB1. GPRPA1 is expressed significantly lower in the sera of PRL patients than the control group and knockdown of GPRPA1 negatively regulates cell motility in the placental cell. Therefore, down-expressed GPRPA1 would be one of the causes of RPL and can be utilized as a serum biomarker of RPL.
Limitations, reasons for caution: Additional *in vivo* study is needed to specifically investigate the effect of GPRPA1 on the pathogenesis of RPL.
Wider implications of the findings: By investigating the cellular functions of GPRPA1 in the placental cell, we found that it is an important key factor for the pathogenesis of RPL, and down-regulation of GPRPA1 can be utilized as a biomarker of RPL.
Trial registration number: not applicable

P-422 Acylglycerol Kinase is a new novel marker in preeclampsia

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Study question: What is the potential function of Acylglycerol Kinase (AGK) in the pathogenesis of preeclampsia(PE).
Summary answer: AGK plays an important role in the pathogenesis of PE by influencing the function of the trophoblast cells.
What is known already: PE is the leading cause of maternal and perinatal mortality and morbidity. The underlying mechanism is still not completely elucidated. Disorder migration, invasion and mitochondria function of trophoblast cells are one of mechanism in preeclampsia. AGK is a subunit of the mitochondrial channel protein complex TIM22, and maintain the stability of mitochondrial structure and function. Studies have shown that AGK is related to the development of various cancers by infecting the cells migration and invasion. It will be interesting to explore the potential function of AGK in the process of early trophoblast development and the pathogenesis of PE.
Study design, size, duration: Firstly, explore the expression of AGK in PE. Secondly, lentivirus systems was used to generate loss and gain of function models in trophoblast cell line-HTR8/Sneov to study the role of AGK in the pathogenesis of PE.

Participants/materials, setting, methods: We examined the expression of AGK both in placental tissues from PE patients and normal pregnant patients. Meanwhile, we generated AGK loss and gain of function models in HTR8/Sneov cells by using lentivirus systems. Transwell assays, scratch-wound assays, EDU and plate clone formation assays, cell apoptosis assays, cell cycle assays, ATP concentration, mtDNA level and transmission electron microscopy were used to examine the function of AGK in HTR8/Sneov cells model.
Main results and the role of chance: In this study, AGK was significantly decreased in the extra-villous trophoblast (EVT) cells in placental tissues of PE patients compared with that in control group by immunohistochemistry. And further confirmed the expression of AGK in placental tissues by QPCR, western blot. We demonstrated that knockdown of AGK in HTR8 dramatically decreased the cell proliferation, migration and invasion. It also showed the significantly lower plate clone formation rate in AGK knockdown -HTR8 cells compare with the WT HTR8 cells. While overexpression of AGK in HTR8 dramatically increased the cell proliferation, migration, invasion and higher plate clone formation rate. Further, we demonstrated that AGK regulated the ATP level and mtDNA level in HTR8 cells model. And we found that knockdown AGK decreased the number of mitochondria, and shown the mitochondrial crista disorder, mitochondrial swelling and dissolution and mitochondrial membrane fragmentation. While overexpression AGK increased the number of mitochondria.
Limitations, reasons for caution: Although we show that AGK played an important role in the function of HTR8, the study of working mechanism of AGK in PE is still very limited. More studies will be performed to explore its underline mechanism.
Wider implications of the findings: This study was the first time to explore the role of AGK in PE. It will help us to better understand the pathogenesis of PE, which might be helpful in future application of novel therapeutic targets in PE.
Trial registration number: not applicable

P-423 Lower cytotoxicity: Altered natural killer cell activation in recurrent implantation failure

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Study question: Within this prospective study, we aim to differentiate immune cell subpopulations in recurrent implantation failure (RIF) patients and fertile controls.
Summary answer: A misbalanced immune profile of NK cell subpopulations is present in RIF patients and might be a potential risk factor that requires further detailed analysis.
What is known already: So far, there is no conclusive opinion on the prognostic value of testing immune cell populations in women with RIF. Increased numbers of cytotoxic (CD56dimCD16bright) peripheral natural killer (pNK)-cells and CD56brightCD16dim mainly in the uterus occurring NK cells (uNK) seemed to be more prevalent in RIF patients. NK cell cytotoxicity is regulated by a complex interaction of activating and inhibiting receptors, such as the NKGD2 and natural cytotoxicity receptors including NKp46, NKp30 and NKp44. Dysregulated pNK cells could affect the adhesion and implantation of the embryo thereby contributing to RIF.
Study design, size, duration: Within this prospective study between March 2018 and August 2020 immune diagnostics of pNK cells and subpopulations as well as regulatory T-cells in RIF patients (defined as ≥ 3 failed fresh or frozen embryo transfers of good quality embryos (Istanbul criteria) and non-pregnant controls (nulli- and multipara) were performed using flow cytometry analysis.
Participants/materials, setting, methods: In total, n = 42 RIF and n = 85 controls were included. Absolute numbers and percentages of total lymphocytes of CD56dimCD16bright, CD56brightCD16dim NK cells, CD45+CD25+FoxP3+-regulatory T-cells and activation markers (CD57+, CD62L+, NKGD2+, NKp46+) were measured in patients and controls (n= 60 nulligravida, n= 25 para) in the mid-luteal phase. Statistical analysis was performed using SPSS Version 26 considering $p < 0.05$ statistically significant.
Main results and the role of chance: RIF patients showed significantly lower numbers and percentages of CD56dimCD16bright pNK cells (mean±SD per µl: 187,5±113,3 vs. 281,9±163,4 p=0.001; %: 87,4±8,8 vs. 90,6±6,0 p=0.017) and higher levels of CD56brightCD16dim pNK cells (mean±SD per %: 10,5±8,3 vs. 7,6±5,5 p=0.021) compared to controls. Further, lower percentages of CD56dimCD16brightCD62L+ (mean±SD per %: 23,5±11,1 vs. 32,0±14,0 p=0.001), CD56dimCD16brightNKGD2+ (mean±SD per %: 94,0±6,8 vs. 96,4±4,2 p=0.014) and CD56dimCD16brightNKp46+ (mean±SD per %: 65,8±19,5 vs. 76,1±14,0 p=0.001) were observed in RIF patients ($p < 0.05$). A different activation of pNK cells represented by high levels of CD62L+, NKGD2+, NKp46+ surface markers in controls and higher levels of CD56brightCD16dim pNK cells in RIF patients could contribute to RIF. No difference was present in levels of CD45+CD25+FoxP3+-regulatory T-cells within the study population.
Limitations, reasons for caution: As controls composed out of not only nulli- but also multipara, higher levels of pNK cells in controls, could be induced by fetal microchimerism in multiparas, however, results remained significant after removing multipara from statistical analysis.
Wider implications of the findings: These findings condense into the assumption of a non-linear association between NK cytotoxicity and successful pregnancy. A lower NK cytotoxicity in RIF patients could potentially lead to an altered immune environment impeding a successful implantation process.
Trial registration number: Drks00020803

P-424 Evaluating the relationship between endoplasmic reticulum stress and recurrent pregnancy loss in a young population cohort

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Study question: Does endoplasmic reticulum (ER) stress evaluated by X-box binding protein 1 (XBP-1) among patients aged 18-30 years play a role in recurrent pregnancy loss (RPL)?
Summary answer: High levels of XBP-1 observed in patients with known RPL indicate that ER stress plays an important role in RPL.
What is known already: Female reproductive tract undergoes dynamic changes during oogenesis which require protein synthesis, folding, maturation, which take place in the ER. ER is also responsible for destruction of unfolded/misfolded proteins. Excess accumulation of these faulty proteins leads to ER stress, which actives unfolded protein response (UPR). XBP-1 is a transcription factor involved in UPR and regulates ER stress-mediated apoptosis. Regulation of ER homeostasis is important in folliculogenesis, oocyte maturation and embryogenesis. It is also known that ER stress has a positive correlation with age and it is associated with age-related diseases.
Study design, size, duration: This prospective case-controlled study was conducted at University of Health Sciences Turkey, Istanbul Kanuni Sultan Suleyman Training and Research Hospital Department of Obstetrics and Gynecology between March 2020 – September 2020. A total of 70 subjects were included in the study. All patients gave their written informed consent before their enrollment in the study.
Participants/materials, setting, methods: 38 patients aged 18-30 years with a history of RPL were included in the study. Patients who had miscarriages due to fetal abnormalities, patients with infections, endocrine or genetic disorders, smokers, alcohol and/or drug abusers, with acute/chronic inflammatory diseases, patients using steroids, anti-inflammatory and antioxidant medications were excluded from the study. Age-matched 32 healthy subjects without RPL were included in the control group. XBP-1 levels were determined using Human XBP-1 ELISA Kit (Elabscience Co., USA).
Main results and the role of chance: The mean age in the control group and in the study group were 25.21±3.3 and 25.26±2.6, respectively and they

were statistically similar (p = 0.324). When groups were compared according to thyroid stimulating hormone (TSH) levels and body mass index (BMI), which are additional risk factors of RPL both groups were statistically similar (p = 0.642 and 0.942, respectively). As expected gravidity and abortus numbers were significantly higher in the study group (p <0.001). A mean XBP-1 level of 1233.41± 3902.97 was determined in the control group. The mean value of the study group was calculated to be 2251.49± 9621.12. Mean XBP-1 level in the study group was significantly high (p <0.001). A receiver operating curve (ROC) analysis was conducted in the study group. The area under the curve was found to be 87% (95% CI: 79% - 95%). The specificity was 75%, sensitivity was 89%, positive LR was 3.5, negative LR was 0.15, positive predictive value was 80% and negative predictive value was 87% for the cut-off XBP-1 level at 1364.68 pg/mL.

Limitations, reasons for caution: Small sample size is an important limitation of this study. In addition, evaluating XBP-1 only in serum samples does not let us drive any conclusions on the local changes of ER stress. Studies with larger samples sizes and studying XBP-1 levels in tissue samples of endometrial material is needed.

Wider implications of the findings: The significantly high levels of XBP-1 in RPL patients younger than 30 years, indicate higher ER stress in this group even when age dependent increase in ER stress is calculated out of the equation. XBP-1 can be a promising marker in evaluating patients with a fertility wish for RPL risk.

Trial registration number: NCT04455256

P-425 Recurrent pregnancy loss: what can we learn from different international guidelines?

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Study question: To which extent do the current international guidelines and recommendations concerning recurrent pregnancy loss (RPL) differ?

Summary answer: All guidelines apply definitions for RPL, however few diagnostic and therapeutic options are described. Diagnostics should be based on best evidence and current scientific knowledge.

What is known already: Established risk factors for RPL include anatomical, genetic, endocrine, hemostatic and immune alterations. The European Society of Reproduction and Embryology (ESHRE), American Society of Reproductive Medicine (ASRM), German/Austrian/Swiss Society of Obstetrics and Gynecology (DGGG/OEGGG/SGGG) and the Royal College of Obstetricians and Gynecologists (RCOG) published guidelines concerning diagnostic and therapeutic options in RPL. Due to the different guideline processes and date of publication actuality as well as complexity differ widely.

Study design, size, duration: We compared the guidelines of the ESHRE, ASRM, DGGG/OEGGG/SGGG and RCOG with regard to definition, diagnostic and therapeutic aspects. The guidelines were published between 2011 and 2018. Structured guideline processes with regular (complete) updates are only provided by the DGGG/OEGGG/SGGG.

Participants/materials, setting, methods: After thorough literature research (Pubmed, Embase) all existing guidelines and recommendations were analysed and compared considering the current state of knowledge. The RCOG recommendations from 2011 were updated in 2014 and 2017, the ARSM expert letter was last updated in 2012. The ESHRE guideline was published in 2017. The first version of the DGGG/OEGGG/SGGG guideline was published 2006, updated in 2013 and upgraded to a higher evidence-level in 2018 and is currently under review.

Main results and the role of chance: All guidelines agree that a diagnostic work-up is indicated after at least two clinical pregnancies and should exclude anatomical malformations, an antiphospholipid syndrome and thyroid dysfunction. Furthermore, lifestyle modifications are recommended by all. The general evaluation of an inherited thrombophilia is not recommended by any guideline. Exclusion of other risk factors like parental chromosomal disorders, a polycystic ovary syndrome or insulin resistance are only included in some guidelines, partly due to a lack of diagnostic criteria (luteal phase insufficiency) or due to the different year of publication of the recommendations (e.g. chronic endometritis). All guidelines recommend treating APLS by administering low-dose aspirin (75-100mg daily) in combination with unfractionated/low-molecular-weight heparin. With regard to uterine malformations whether or not a septum should be dissected is still a matter of debate: ESHRE and RCOG consider evidence insufficient, while DGGG/OEGGG/SGGG and ASRM recommend a surgical intervention. In case of chronic endometritis, the DGGG/OEGGG/SGGG recommends antibiotic therapy e.g. with doxycycline (200 mg daily for 14 days).

Limitations, reasons for caution: Different health economic as well as consensus aspects in the process of guideline development have a significant influence on the individual guidelines and recommendations.

Wider implications of the findings: Since personalized diagnostic and therapeutic strategies in RPL patients are required, physicians have to decide when to follow the guideline and when to expand diagnostics and therapy. Therefore, the knowledge of the weaknesses of each guideline and its developmental process is helpful for treating RPL couples.

Trial registration number: -

P-426 Uterine Natural Killer Cells in Recurrent Miscarriage and Implantation Failure: An Updated Systematic Review and Meta-analysis

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Study question: Do women with recurrent miscarriage (RM) or implantation failure (RIF) have different levels of uterine Natural Killer (NK) cells compared to fertile controls?

Summary answer: Women with RIF but not RM are associated with significantly higher levels of CD56+ uterine NK cells compared to controls.

What is known already: Uterine NK cells (uNK) are different from peripheral NK cells (pNK) and are important in early pregnancy for development of the placenta. The association between uNK and RM/RIF is less clear, but dysfunction of uNK is believed to result in early pregnancy failure. Previous systematic reviews by Seshadri (2014) and Tang (2013) on infertile and RM patients showed no significant difference in uNK levels and highlighted need for further studies. Since, many prospective studies have been published and therefore warrant an updated systematic review. On the other hand, evidence for correlation between uNK and pNK is sparse and needs clarification.

Study design, size, duration: We have conducted a systematic review and meta-analysis to evaluate three outcomes. The primary outcome was the difference of uNK level in RM/RIF compared to controls. The secondary outcome was livebirth rate in women with RM/RIF with high compared to normal uNK level, and the tertiary outcome was correlation between uNK and pNK in RM/RIF.

Participants/materials, setting, methods: The electronic database search included MEDLINE, EMBASE, Web of Science and bibliographies from included articles from inception to December 2020 using a combination of MESH and keywords. Search, screen, and data extraction were performed by two reviewers independently. Quality assessment was conducted with ROBINS-I and meta-analysis with Revman 5.3. Out of 4636 studies screened, 43 studies (2539 women) and 3 studies each (598 and 77 women) were analysed for primary, secondary and tertiary outcomes respectively.

Main results and the role of chance: Our meta-analysis showed that CD56+ uNK were significantly higher in women with RIF but not RM compared to controls (SMD 0.60; 95% CI 0.12-1.08]. Subgroup analysis in RM patients showed no significant difference whether definition of 2 or 3 previous RM was used, in primary/secondary RM compared to controls, or in primary versus secondary RM. CD56+ uNK were significantly higher in RM/RIF when sampled during mid-luteal phase [SMD 0.56; 95% CI 0.19-0.93] but not in the early pregnancy decidua. Interestingly, there was significant difference in CD56+ uNK when analysed by immunohistochemistry [SMD 0.50; CI 0.05-0.94] but not by flow cytometry, and when CD56+ uNK were reported as percentage over total endometrial cells [SMD 0.58; 95% CI 0.10-1.07]. Further subgroup analysis showed significant difference in CD16+ [SMD 0.54; 95% CI 0.18-0.89] but not in CD56+CD16-, CD56+CD16+ or CD57.

For pregnancy outcome, there was no significant difference in livebirth rate in RM/RIF patients with high uNK compared to normal uNK [RR 1.06, 95% CI 0.86-1.30]. Mean uNK level in RM patients with subsequent miscarriage was not significantly higher than subsequent livebirth.

Finally, the pooled correlation between CD56 pNK and CD56 uNK (r=0.42; 95% CI -0.04-0.73] was not significant in RM/RIF patients.

Limitations, reasons for caution: The meta-analysis is limited by quality of some of the studies. Some data were presented in median that was transformed to mean which may result in data skew. Other confounding factors e.g. maternal age, fetal karyotype, number of previous miscarriages and variable definition of controls may contribute to bias.
Wider implications of the findings: Clinical interpretation of uNK level needs to be treated with caution because there is significant heterogeneity in method of analysis. There may be a role for uNK measurement in RIF patients however further studies to understand pathophysiology underlying elevated uNK is warranted before recommending it as a diagnostic tool.
Trial registration number: N/A

P-427 Maternal and fetal thrombophilia mutations and the ANXA5 M2 haplotype are more prevalent in first trimester miscarriages of euploid embryos

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Study question: Is there a cumulative effect of shared maternal and fetal thrombophilia mutations and the ANXA M2 haplotype in first trimester miscarriages and are there shared genotypes that may predict outcome?
Summary answer: Coexistence of MTHFR-677CT and PAI-1-4G/5G-mutations in euploid-fetus-mother -pairs may adversely affect early development. Maternal FII-20210GA and PAI-14G mutation were more prevalent in women who miscarried euploid-fetuses.
What is known already: Spontaneous abortion (SA) is a significant clinical problem with several different etiologies. Certain thrombophilia and folate-related gene mutations have been associated with an increased risk of miscarriage which may be due to impaired coagulation homeostasis. The M2-haplotype of ANXA5-gene promotor encoding the anticoagulation protein Annexin-A5 has also been implicated in early and recurrent SA. However, one important deficiency in the majority of the studies on this subject is a lack of information on the fetal/embryonic contribution to the thrombotic events during placentation and early embryo development. The purpose of this study was to evaluate the prevalence of specific and cumulative thrombophilia mutations in women experiencing first trimester (T1) miscarriages and in their fetal products of conception.
Study design, size, duration: This is a single Centre retrospective cohort study where we analyzed the frequency and cumulative effect of mutations in 10 thrombophilia/folate related genes and the ANXA5 M2 haplotype in first trimester miscarriages from 229 mother-product of conception (POC) pairs from Jan. 2018-Nov. 2020.
Participants/materials, setting, methods: All 229 POC DNA samples were confirmed to be fetal and had aneuploidy screening with NGS. We analyzed 10 different mutations in thrombophilia- and folate-related genes (Factor V-Leiden G1691A, Factor V-H1299R, Factor II-G20210A, Factor XIII-V34L, PAI-I-675 4G/5G, FGB-455G/A, MTHFR-C677T and -A1298C, MTR-A2756G, and MTRR- A66G) using single base sequencing methodology and the M2 haplotype of ANXA5 promoter by Sanger sequencing. Maternal–euploid fetus pairs were compared to maternal-aneuploid fetus pairs. SSPS software was used for statistical analyses, and $p<0.05$ with CI 95% was considered significant.
Main results and the role of chance: Aneuploidy was detected in 40.6% (93/229) of POC, and the rest were euploid. Women with euploid fetuses had a significantly higher FII20210GA mutation frequency than those who had aneuploid fetuses ($p=0.02$). However, this difference was not detected in the fetuses. Minor allele frequency and genotype frequencies of MTHFR A1298C, MTR A2756G and MTRR A66G mutations as well as for Factor V-Leiden G1691A, Factor V-H1299R and mutations were similar between the groups studied. The frequency of the M2 ANXA5 haplotype was similar between the two groups (3.33% maternal-euploid fetus-pairs vs 3.1% in maternal-aneuploid fetus pairs). PAI-I -675 4G/4G genotype was more frequent in women with euploid fetal losses (25%) vs aneuploid (15%) ($p=0.05$), but with no-significant difference in euploid (20%) vs aneuploid fetuses (13%). A cumulative effect of maternal and fetal mutations in MTHFR 677CT and PAI-14G was observed in maternal-euploid fetus pairs ($p=0.0011$, OR 3.8 95%CI [1.8-11.3], and $p=0.05$, OR 1.2 95%CI [1.12-3.6], respectively). Overall mutation load in tested samples was 4.14 mutations±1.5 [1.46±0.8-thrombophilia and 2.45±1.16-folate metabolism] and was similar between both groups of fetuses and their mothers.
Limitations, reasons for caution: The retrospective nature and cohort design limited the generalizability of our results. The comparison group is maternal-aneuploid fetus- pairs as the reason for the miscarriage is the chromosomal abnormality in the fetus. The ethnic background may be a cofounding factor that needs to be evaluated in a larger study which is ongoing in our center.
Wider implications of the findings: Our study found a high mutational load in the 10 folate metabolism and thrombophilia genes we studied in the maternal-fetus pairs. Our findings provide evidence that fetal contribution to placental thrombophilia should be considered in cumulative effect estimations of specific maternal mutations in improvement of management of early miscarriages.
Trial registration number: not applicable

P-428 Uterine infusion of autologous platelet rich plasma (PRP) before embryo transfer may improve the transfer outcomes in recurrent implantation failure and thin/scarred endometrium patients

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Study question: Does the infusion of autologous platelet rich plasma (PRP) to the uterus improve the outcomes of embryo transfer of thin endometrial or recurrent implantation failure (RIF) patients?
Summary answer: Autologous PRP uterine infusion may improve the result of embryo transfer (ET) in RIF group and thin/scarred endometrial group.
What is known already: Autologous PRP has been proposed to improve the outcomes of various treatment procedures. In infertility, several trials have reported an improvement in endometrial thickness in patients having thin endometrium thus previously cancelled ET cycles. Uterine injection of PRP shortly before ET has been proposed to improve the results of ET in patients having RIF. Platelets in PRP would be activated via different pathways to release growth factors and cytokines. In this study, we applied our in-house-developed PRP extraction kits that use a mechanical activation/platelet breaking down method to infuse/inject into the uterine of the poor prognosis transfer patients.
Study design, size, duration: This study includes two phases: Phase 1 (04/2019-12/2019): we tested the safety and effectiveness of the PRP extraction kits in 30 volunteers regardless of the gender by derma-rolling process using PRP extracted by our kits. Phase 2 (02/2020-12/2020): 111 IVF patients who had thin/scarred endometrium previously having at least one cancelled ET cycle (group 1) or patients who had at least two implantation failure ETs (group 2) were enrolled in the study.
Participants/materials, setting, methods: 20 mL blood was drawn from the vein. After centrifugation, PRP was filtrated through a filter to break down platelets releasing growth factors/cytokines. Firstly, 30 volunteers (average age of 34.4 ± 5.5) were derma-rolled on the facial skin twice (one week apart). Secondly, IVF group 1 was uterine-infused with 0.5 mL PRP on day 7/8 of the ET cycle, both groups were uterine-infused with 0.5 mL PRP two days (40-48 hours) before ET.
Main results and the role of chance: 0.5 mL of PRP before filtering was measured and calculated to have 8-12 folds increase of platelet concentration. In phase 1, no side-effects or complications were recorded. The average skin pore size reduced by approximately 0.01 mm2 in all patients two weeks after treatment. In phase 2, the average age was 35.6 ± 6.1. Group 1 had 31 patients and group 2 had 99 ones. In group 1, five patient did not obtained improvement in endometrium then ET cycles were cancelled, one patient did not have blastocyst to transfer and 25 patients had endometrium of at least 7 mm thick before ET and ET (100 frozen ET) were carried on. One couple was not contactable. Out of 24 couples, 13 had biochemical pregnancy (54.2%) and 11 had clinical

pregnancy (44.0%). Group 2 had 80 patients. One of them did not have embryo to transfer. 37/79 embryo transfers had biochemical pregnancy (46.8%) and 44.3 % clinical pregnancy. No complication was recorded. In our cohort, several successful patients had more than 7 unsuccessful ETs previously. For reference, in 2020, our clinic had 4260 ETs in total, the clinical pregnancy rate was 60.1%, the average age was 31.82 years old.

Limitations, reasons for caution: Each case in phase 2 of this study had a complicated fertility medical history therefore it was impossible to select the control group. This study is descriptive only. The size of each group was relatively small requiring ongoing data recording.

Wider implications of the findings: This study support the idea that cytokines and growth factors in PRP may help to prepare endometrium for ET, safely and effectively.

Trial registration number: Not applicable

POSTER VIEWING
MALE AND FEMALE FERTILITY PRESERVATION

P-429 Calcium analysis and embryonic development of in vitro matured oocytes from transgender men

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Study question: Can oocytes isolated from transgender men after oophorectomy support embryonic development?

Summary answer: Embryo developmental arrest at 4-8cell stage (day 3 embryos) indicates poor quality of *in vitro* matured oocytes from transgender men.

What is known already: Gender affirming surgery for transgender men leads to permanent infertility, as it involves bilateral oophorectomy. Current approaches for fertility preservation, such as oocyte freezing following ovarian hyperstimulation, may interfere with the wanted masculine characteristics and enhance gender dysphoria. *In vitro* matured oocytes (IVM) isolated following oophorectomy have been proposed as a source of potential gametes to ensure fertility preservation for transgender men with a child wish. From previous studies, it has been shown that these oocytes are able to undergo maturation and display normal spindles, but their competence to be fertilized and support embryonic development has not been addressed yet.

Study design, size, duration: We evaluated the quality of *in vitro* matured oocytes isolated from ovaries of transgender men by applying calcium imaging and monitoring fertilization and embryonic development following intracytoplasmic sperm injection (ICSI). Ovaries were collected in cold (4oC) or warm (37oC) medium, to investigate the best collection procedure. So far, results from four transgender men have been included. Participants/materials, setting, methods: Ovaries from four transgender men undergoing testosterone treatment were collected after oophorectomy in cold or warm medium. Cumulus oocyte complexes (COCs) were isolated and cultured in maturation medium for 48hrs. Mature oocytes were injected with donated sperm and assessed either by calcium imaging, measuring the total calcium release following injection, or following embryonic development. Donated *in vitro* matured oocytes, germinal vesicle(GV) or metaphase I(MI) origin, from other patients undergoing IVF treatment were used as controls.

Main results and the role of chance: In total, 179 COCs were collected from ovaries (n=8) of four transgender men. From the COCs collected in warm medium, 73/105(69%) survived and 33/73(45%) reached metaphase II (MII). Of 21 MII injected with sperm, 13/21(62%) fertilized, 9/21(43%) formed 2 pronuclei (PN), 8/9(89%) reached the 2-cell stage, 3/9(33%) reached 4-8cell stage but arrested. From 74 COCs isolated in cold medium, 57/74(77%) survived and 28/57(49%) matured. Of the 11 MII injected with sperm, 7/11(64%) fertilized, 6/11(54%) formed 2PN, 6/6(100%) reached the 2-cell stage, 4/6(67%) reached 4-8cell but arrested. In the control group, 10/13 oocytes injected with the same sperm sample, were normally fertilized (77%), 8/10(80%) reached the 2-cell stage, 7/10(70%) reached the 4-8cell stage and 4/10(40%) became blastocysts. From the warm, cold and control conditions, respectively 12,14 and 17 MII oocytes were used for calcium imaging. The product of amplitude and frequency of calcium peaks, representing total calcium release, was calculated. Oocytes showed an average release of 0.66AU and 1.69AU for the warm and cold condition, respectively. The average value for control oocytes was 2.19AU.

Limitations, reasons for caution: One major limitation of our study is the lack of ovaries from cis women as control group. Our control oocytes originated from women undergoing IVF treatment and have undergone ovarian stimulation. Furthermore, the number of oocytes analysed and number of patients per group was limited and is being increased.

Wider implications of the findings: Our data indicate that *in vitro* matured oocytes from transgender men ovaries display poor quality, as demonstrated by the poor embryonic development. In the future, we will apply nuclear transfer technology as a mean to overcome embryonic developmental arrest in this group of oocytes.

Trial registration number: Not applicable

P-430 Male fertility preservation: is there a role for cancer-induced inflammation that affects semen quality in oncological patients?

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Study question: What is the cause of semen quality impairment in oncological patients during fertility preservation programs? The cancer type and stadiation or the resulting inflammatory state?

Summary answer: The inflammatory state seems to be related to the decrease of sperm concentration, motility, morphology and viability due to the worsening of oxidative stress microenvironment.

What is known already: Fertility preservation acquired a great importance in the last decades due to increase survival of oncological patients, boost of diagnosis under 40 years and postponement of paternal age. At the time of cryopreservation, only one third of these males are normozoospermic. Tumor itself or other factors, added to psychological reasons, may be involved but there is no clear evidence. An imbalance of ROS (reactive oxygen species) in semen can compromise its quality. However, the correlation between cancer-related generalized stress state and fertility is poorly investigated. Inflammatory conditions induced by infections and pathologies, including cancer, increase ROS.

Study design, size, duration: Retrospective observational analysis was performed on 45 patients (29.0 ± 6.9 yrs) recruited during their fertility preservation program between 2016 and 2019 with written consent on use of their clinical data for research purpose. Patients presented several oncological diagnoses. Semen samples obtained from multiple collections (N=58) were analyzed before applying standard freezing protocol. Data on semen parameters, inflammatory indices, hematological values and type/stage of tumors were collected. No exclusion criteria were applied.

Participants/materials, setting, methods: Routine semen analysis was performed according to the WHO standards. Sperm concentration and motility were evaluated on Makler Chamber, whereas eosin stain and Diff-quick slides were used for viability and morphology, respectively. Lymphoma was present in 72% of cases, leukemia in 8%, seminoma in 7% and other cancers in 13%. Correlations (Pearson/Spearman tests) among principal semen parameters and hematological values (leukocytes, erythrocytes, hemoglobin, RDW, albumin, etc.) were calculated with a P-value <0.05 considered statistically significant.

Main results and the role of chance: The majority of semen samples showed a severe impairment, with one or more parameters under lower reference limits (WHO): 48.3% had sperm concentration under 15 millions/ml, 43.1%

had a progressive motility under 32%, 41.4% had viability under 58% and 91.4% had abnormal morphology (under 4%). The role of potential inflammatory state was analyzed by correlating semen parameters and some hematological values. No correlation was found with cancer type. Negative association resulted between progressive motility (%PR) and leukocytes (p=0.041) or RDW% (p=0.015), but positive one with albumin (p=0.012). Even sperm count, total motility (%PR+NP) and morphology were significantly correlated to RDW% (p=0.003, p=0.032, p=0.034, respectively). These findings suggest a possible role of inflammation and ROS related generation in semen quality impairment. Indeed, albumin exerts a protective action, but leukocytes are known to cause ROS increase. Cancer-induced oxidative stress state may alter red blood cells homeostasis and vitality and increase erythrocytes turnover resulting in high RDW values. It is likely semen is worse when blood values indicate more severe cancer-induced inflammatory condition.

Limitations, reasons for caution: Significant correlations with type/stage of cancer were not found due to small number of each diagnosis, in spite our study considered 3 years of patients inclusion. Moreover, we lack to analyze the same patient before the cancer onset to avoid the influence of inflammatory state generated by the tumor itself.

Wider implications of the findings: Understanding the influence of cancer-induced inflammatory state on semen quality could increase the awareness that clinicians should direct patient to the fertility preservation as soon as possible, even if diagnosis is still ongoing. It should be evaluated whether offering specific treatments may reduce oxidative stress conditions.

Trial registration number: Not applicable

P-431 Human oocytes in-vitro maturation efficacy from infancy to adulthood – is there an optimal age?

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Study question: Does human oocytes in-vitro maturation (IVM) effectiveness change throughout childhood, adolescence and adulthood in girls and women undergoing fertility preservation via ovarian tissue cryopreservation (OTC) prior to chemo-radiotherapy exposure?

Summary answer: The optimal age for IVM is from menarche to 25 years, while pre-menarche girls and women older than 30 years have extremely low maturation rates.

What is known already: In vitro maturation of oocytes from antral follicles seen during tissue harvesting is a fertility preservation technique with potential advantages over OTC, as mature frozen and later thawed oocyte used for fertilization poses decreased risk of malignant cells re-seeding, as compared to ovarian tissue implantation. We previously demonstrated that IVM performed following OTC in fertility preservation patients, even in pre-menarche girls, yields a fair amount of oocytes available for IVM and freezing for future use.

Study design, size, duration: A retrospective cohort study, evaluating IVM outcomes in chemotherapy naïve patients referred for fertility preservation by OTC that had oocyte collected from the medium with attempted IVM between 2003 and 2020 in a university affiliated tertiary center.

Participants/materials, setting, methods: A total of 133 chemotherapy naïve patients aged 1-35 years with attempted IVM were included in the study. The primary outcome was IVM rate in the different age groups – pre-menarche (1-5 years and ≥6 years), post-menarche (menarche-17 years), young adults (18-24 years) and adults (25-29 and 30-35 years). Comparison between paired groups for significant difference in the IVM rate parameter was done using the Tukey's Studentized Range (HSD) Test.

Main results and the role of chance: A gradual increase in mean IVM rate was demonstrated in the age groups over 1 to 25 years (4.6% (1-5 years), 23.8% (6 years to menarche) and 28.4% (menarche to 17 years), with a peak of 38.3% in the 18-24 years group, followed by a decrease in the 25-29 years group (19.3%), down to a very low IVM rate (8.9%) in the 30-35 years group. A significant difference in IVM rates was noted between the age extremes – the very young (1-5 years) and the oldest (30-35 years) groups, as compared with the 18-24-year group (p<0.001). Number of oocytes matured, percent of patients with matured oocytes and overall maturation rate differed significantly (p<0.001).

Limitations, reasons for caution: Data regarding ovarian reserve evaluation was not available for most of the patients, due to our pre-op OTC procedures protocol. None of our patients have used their frozen in-vitro matured oocytes, as such further implications of age on in-vitro matured oocytes quality and implantation potential has yet to be evaluated.

Wider implications of the findings: Our finding of extremely low success rates in those very young (under 6 years) and older (≥30 years) patients suggest that IVM of oocyte retrieved during OTC prior to chemotherapy should not be attempted in these age group.

Trial registration number: N/A

P-432 Do stage and grade of malignancy impact fertility preservation in breast cancer patients?

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Study question: Do stage and grade of breast cancer impact the number of retrieved mature oocytes during controlled ovarian stimulation for fertility preservation?

Summary answer: Stage and grade of breast cancer do not impact the number of retrieved mature oocytes. Higher grade breast cancer requires higher gonadotropin doses during stimulation.

What is known already: Cancer can impair ovarian response by unknown mechanisms. Some authors suggest that it could be detrimental on fertility because it elicits a catabolic state, increasing stress hormone levels. Some studies have also shown that ovarian response to controlled ovarian stimulation (COS) is, in some way, compromised in oncological patients. Little is known about the impact of different types of cancer on ovarian reserve, and specifically whether higher stage and grade could compromise egg retrieval during fertility preservation (FP) techniques. Study design, size, duration: Retrospective cohort study evaluating data of FP treatment cycles among women with breast cancer at the Oncofertility Unit of San Raffaele Hospital, Milan in the period from 2011 to 2019.

Participants/materials, setting, methods: Inclusion criteria were: breast cancer diagnosis; age 22-41; oocyte cryopreservation after stimulation with a random start GnRH-antagonist protocol. Patients receiving chemotherapy before FP were excluded. We compared outcomes between low-stage (stage I) and high-stage (stage II-III) patients and low-grade (G1-G2) and high-grade (G3) patients. Main study outcome was the total number of retrieved mature oocytes. Univariate analysis was performed by Mann-Whitney test, Kruskal-Wallis test and Fisher's exact test. Multivariate analysis was performed by logistic regression.

Main results and the role of chance: 101 stimulation cycles were included. High-stage disease patients were significantly younger than low-stage. Median antral follicle count (AFC) was 12 in low-stage and 10 in high-stage (age-adjusted p=0.92) and median anti-mullerian hormone (AMH) levels were 1.9 ug/L in low-stage and 1.8 ug/L in high-stage (age-adjusted p=0.22). No significant difference in stimulation protocols and follicle-stimulating hormone (FSH) start and total dose could be detected between the 2 groups. Median number of vitrified oocytes was 7 in both groups (p=0.75). No significant difference could be observed in median AFC (13 vs 10, p=0.14) and AMH levels (2.1 vs 1.5, p=0.88) between low-grade and high-grade disease patients. When adjusting for age, AFC was found to be significantly lower in high-grade disease patients (p=0.03). Patients with high-grade tumors were stimulated with higher doses of FSH (age-adjusted p-value=0.03). Median number of vitrified oocytes was 6 in low-grade patients and 7 in high-grade (p=0.35). In a multivariate model including age, cancer stage, cancer grade and molecular classification, the only significant factor found to be inversely associated with AFC was cancer grade (OR 3.6; 95% CI 0.7 – 6.5, p=0.01), while only age was significantly associated with oocyte retrieval (OR 0.4; 95% CI 0.01 – 0.9, p=0.04).

Limitations, reasons for caution: The main limitations of our study are its retrospective design and the small sample size.
Wider implications of the findings: Fertility preservation counselling and ovarian stimulation protocols of breast cancer patients could be implemented with cancer grade.
Trial registration number: not applicable

P-433 Pregnancy and livebirth after fertility preservation in cancer patients

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Study question: What do we know about pregnancy and livebirth after IVF-fertility preservation treatment in women with cancer?
Summary answer: Most women conceived spontaneously (60%) and more than 50% of those who returned to use their cryopreserved reproductive material have delivered at least one child.
What is known already: Diminishing ovarian reserve and declining future reproductive potential are important issues in cancer survivors after anti-cancer treatment exposure. Publications on pregnancy and livebirth after fertility preservation in women with cancer are sparse. Studies report most cancer patient who underwent fertility preservation do not come back and use their frozen reproductive material. The purpose of this study was to investigate the fertility preservation outcome among cancer survivors.
Study design, size, duration: A retrospective cohort study was conducted at a single academic fertility center from including 336 cancer patients who underwent IVF-fertility preservation from January 2009 to June 2020.
Participants/materials, setting, methods: We included all women with cancer aged ≤40 years old who were referred for fertility preservation treatment prior to chemotherapy.
Primary outcome: Number of pregnancies and livebirths after spontaneous conception and/or using their stored frozen material.
Secondary outcomes: We also evaluated the utilization rate of the stored reproductive material and mortality rate among those with follow up data.
Main results and the role of chance: Of 336 patients who underwent IVF-fertility preservation, 214 (63.69%) elected oocyte cryopreservation, 86 (25.5%) underwent both embryo and oocyte cryopreservation and 36 (10.7`%) underwent embryo cryopreservation. Follow up data were available in 198 (58.9%) patients with a mean follow up of 3.2 years. Of 198, 16 (8%) patients died, 40 (20%) became pregnant. Of those pregnant patients, 24 (60%) became spontaneously pregnant and 16 (40%) became pregnant after frozen oocyte or frozen embryo treatment cycles. Almost a quarter (72.5%) of the pregnancies resulted in livebirths. In total, only 23 (7%) patients had returned for frozen oocyte or frozen embryo treatment cycle, of which 16 (70%) achieved a pregnancy and 10 (63%) achieved at least one live birth. Of 142 patients who were still alive at follow up but did not get pregnant, 51 (39%) were in remission from their cancer but had not chosen to use their stored reproductive material; 44 (31%) were still on anti-cancer treatment and had not started trying yet; 13 (9%) were suffering from the end-stage cancer disease; and 7 (5%) had used their stored reproductive material but failed and stopped trying to get pregnant.
Limitations, reasons for caution: The main limitation was the retrospective cohort study design which could introduce unidentified selection biases.
Wider implications of the findings: Of women who underwent IVF-fertility preservation for cancer**,** most did not come back for treatment for a variety of reasons. Of those who became pregnant, 60% conceived spontaneously. Of those who used their cryopreserved reproductive material, 63% delivered at least one child.
Trial registration number: 2021/6935

P-434 Effect of let-7a mimic as a new pharmaco-protective agent against chemotherapy-induced ovarian damage on subsequent follicular development and oocyte quality using mouse ovarian transplantation model

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Study question: What is the long-term impact of let-7a-mimic transfection on oocytes development in new-born mice ovaries exposed to chemotherapy in vitro following transplantation in the kidney?
Summary answer: The let-7a-mimic restoration protects against chemotherapy-induced ovarian apoptosis and preserves subsequent follicular developmental and acquisition of oocyte maturation competence in mouse.
What is known already: It is well known that cyclophosphamide and its active metabolites (4-hydroperoxycyclophosphamide, 4-HC) cause irreversible ovarian damage and impair future fertility of cancer survivors. Besides the available fertility preservation options, microRNAs/miRNAs appear to be very attractive and novel targets to prevent theses damage. We showed that miRNAs were dysregulated after exposure to 4-HC in postnatal-day-3 (PND3) ovaries, let-7a being the most downregulated among them. By replacing let-7a function, let-7a-mimic was able to protect mouse follicles against 4-HC in vitro. This previous study suggested that it could preserve the reproductive potential after treatment. However, the impact on subsequent oocytes development is unknown
Study design, size, duration: PND3 ovaries from C57blxCBAF1 hybrid mice were cultured under 3 conditions: control, chemotherapy for 24h (4-HC/20µM/24h), chemotherapy for 24h+let-7a-mimic (4-HC/20µM/24h+let-7a-mimic). Nine PND3 ovaries were cultured in the different conditions and then transplanted under the kidney's capsule of C57blxCBAF1 hybrid adult mice for follicular growth/apoptosis evaluation. Then, 21 ovaries (≥7/condition) were used for oocyte maturation assessment after transplantation and ovarian stimulation. All transplanted mice were observed during 21 days before PND3 ovaries collection. Participants/materials, setting, methods: PND3 ovaries were cultured in vitro using inserts under different conditions. A liposome-based system was used to deliver let-7a-mimic into ovaries and QPCR-assays validated its expression levels after transfection. Apoptosis was evaluated by TUNEL Assay while haematoxylin/eosin staining was used for assessing the follicular morphology, stage and count. The oocyte maturation rate was evaluated at day 21 post-transplantation after gonadotropins injection, mechanical eggs collection and in vitro maturation for 24 hours.
Main results and the role of chance: The apoptosis assessment confirmed that let-7a-mimic transfection reduced the chemotherapy-induced damage in PND3 ovaries in vitro. The number of primordial follicles was significantly reduced ($p<0.05$) compared to control after chemotherapy exposure. However, it was increased in chemo24h+let-7a-mimic compared to chemo24h alone while remaining lower than control ($p>0.05$). Accordingly, the number of the transitory follicles reflecting follicular activation was significantly higher in chemo24h compared to control ($p<0.05$) and chemo24h+let-7a-mimic but for the last one, the result was not significant. Consequently, chemotherapy induces follicle activation while let-7a restoration tends to slow down this effect. To evaluate the long-term effects of chemotherapy and let-7a-mimic transfection, in vitro exposed PND3 ovaries were transplanted under kidney's capsule in female adult mice. After 21 days, the ovarian reserve was higher in control, but we observed a slight increase of follicular density in the chemo24+let-7a-mimic compared to chemo24h. Similarly, the percentage of damaged/apoptotic cells was higher in all chemotherapy exposed groups compared to control but the impact was lower after let-7a restoration (12,0% and 28,2% in chemo24+let-7a-mimic and chemo24h, respectively). Importantly, the oocyte maturation rate after transplantation was higher in chemo24h+let-7a-mimic compared to chemo24h (40% versus 18%, respectively), suggesting a preservation of oocytes maturation competence.
Limitations, reasons for caution: The multiple in vitro/in vivo steps may introduce study bias. Moreover, the oocyte competence and live offspring is currently evaluated. The blastocyst formation and embryo development from oocytes fertilized in vitro, are more relevant parameters for oocyte quality assessment. The birth of healthy animals will confirm let-7a-mimic-transfection safety.
Wider implications of the findings: Our previous study demonstrated the anti-apoptotic effect of let-7a restoration in mouse ovaries against chemotherapy. In the current study, we demonstrated a long-term beneficial effect of let-7a restoration strategy on follicular development and oocytes maturation capacity. The results open new perspectives in fertility preservation using pharmacological approach.

Trial registration number: not applicable

P-435 LH preserves oocyte-granulosa cell communication in mouse ovaries exposed to chemotherapy with alkylating agents

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Study question: Does Luteinizing Hormone (LH) protect the follicular endowment and growth by improving oocyte-granulosa cell (GC) communication of follicles exposed to chemotherapy at the primordial stage?
Summary answer: LH treatment protects mouse primordial follicles against alkylating agents by preventing the chemotherapy-induced follicular depletion and the impairment of oocyte-GC communication during follicular growth.
What is known already: Impaired folliculogenesis is one of the most common deleterious side effects of alkylating agents in ovaries. Bidirectional communication between the oocyte and surrounding GCs is crucial for oocyte development. Therefore, defective gap junctions and reduced oocyte-derived factors compromise folliculogenesis, oocyte competence and meiotic maturation.

Previous findings reported a significant LH protection of follicular viability and meiotic potential of MII oocytes exposed to chemotherapy at primordial stage. Therefore, we aimed to investigate the LH effects on cell junctions and communication between oocyte and GCs in growing follicles derived from quiescent oocytes exposed to alkylating chemotherapy.
Study design, size, duration: Adult 6-week-old CD1 female mice were allocated to control (n=3), chemotherapy (ChT, n=5) and ChT+LH (n=5) groups. Chemotherapy (120 mg/Kg of cyclophosphamide and 12 mg/Kg of busulfan) was intraperitoneally administrated to ChT and ChT+LH mice. ChT+LH animals were pretreated with 1 IU of LH, followed by a second LH dose (1 IU) along with chemotherapy 24 hours later. Control mice only received vehicle (DMSO). Mice were euthanized 30 days later to collect ovaries.
Participants/materials, setting, methods: Follicles were mechanically isolated by puncture with 30-gauge needles from frozen-thawed half-ovaries. Isolated follicles measuring ≥100µm were selected to represent the secondary and later developmental stages. Part of them were kept intact while others were decumulated by using narrow pipettes to obtain denuded oocytes (DOs), and GCs. Follicles, ODs and GCs were analyzed by qRT-PCR to evaluate key factors in oocyte-GC junctions (*Cx37*, *Cx43*, *Cdh1*, *Cdh2*, *Tjp1*) and communication (*Gdf9*, *Bmp15*, *Bmpr2*, *Alk4*, *Alk5*, *Alk6*).
Main results and the role of chance: Chemotherapy induced a 2.1-fold reduction in the number of total isolated follicles (p=0.036), reducing 2.7-fold the number of primordial and primary follicles (<100 µm; p=0.034) and 1.9-fold the amount of growing follicles (≥100 µm; p=0.036) compared to controls. LH-treated ovaries showed a 1.6-fold increase in the total follicle isolation yield when compared to ChT (p=0.032), recovering control-like values (p=ns). This LH protection specially benefited the early-stage follicles (<100 µm), where a 1.9-fold increase in the number of isolated follicles was detected compared to ChT group (p=0.016).

Gene expression analysis of follicles (n=168), DOs (n=110) and GCs (from n=153 follicles) revealed a global downregulation pattern in ChT samples for all genes, when compared to controls, with a significant fold change (FC) reduction for *Gdf9* in follicles (FC: 0.36±0.16); *Cx37*, *Cdh2* and *Gdf9* in DOs (FC: 0.23±0.17, 0.09±0.03, and 0.17±0.07, respectively); and *Cx37*, *Cx43*, *Gdf9* and *Bmp15* in GCs (FC: 0.40±0.23, 0.17±0.07, 0.17±0.08, and 0.04±0.01, respectively). However, LH treated samples showed an overall improvement of gene expression pattern reaching control-like levels for all genes excepting for a downregulation of the *Bmp15* expression in GC (FC: 0.28±0.24; p=0.036).
Limitations, reasons for caution: Animal model study performed with a reduced sample size. Therefore, these findings should be validated in further studies with human tissue samples.
Wider implications of the findings: Our findings suggest that LH treatment prevents the chemotherapy-induced follicle depletion. The LH protection of primordial population seems to preserve its ability to properly establish oocyte-GC interactions during growth and development, which is required to regulate follicular maturation and oocyte competence.

Trial registration number: Not applicable

P-436 Identification of ovarian cell subpopulations by multicolor flow cytometry and its potential impact on ovarian reconstruction programs

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Study question: How could multicolor flow cytometry (MFC) help to identify ovarian subpopulations that could be used for ovarian reconstruction with isolated follicles?
Summary answer: MFC is useful to identify ovarian cell subpopulations in the ovarian cortex.
What is known already: Ovarian tissue cryopreservation is a fertility preservation option for women before gonadotoxic chemo- and/or radiotherapy. However, graft of cryopreserved ovarian tissue must be performed with caution in women suffering from malignancies that may metastasize to the ovaries. For this purpose, functional ovarian tissue qualification is essential to identify ovarian cell subpopulations that could be used for ovary reconstruction in combination with isolated follicles. Furthermore, ischemic tissue damage occurring after the graft is currently another important issue to be resolved for successful ovarian reuse.
Study design, size, duration: We developed an automated ovarian tissue dissociation method to obtain ovarian cell suspensions. Then, we used MFC for the identification of different cell subpopulations in the cell suspension thus obtained.
Participants/materials, setting, methods: Human ovarian tissues from patients undergoing surgery for polycystic ovary syndrome were used in this study. Biopsies of ovarian cortex (fresh or frozen-thawed) were dissociated using an automated dissociation method.

We used FVS780 and SYTO13 markers to gate viable ovarian cells by MFC. Variable markers were chosen to differentiate and identify cell subpopulations among the viable ovarian cells.
Main results and the role of chance: The dissociation yield was on average 1.59 ± 1.58 x 106 and 0.78 ± 0.72 x 106 viable ovarian cells per 100 mg of fresh (n = 17) and frozen-thawed (n = 43) ovarian cortical tissue, respectively. On average, 35.4 ± 13.1 % of viable ovarian cells were CD34+ (n = 61, stromal phenotype). Concerning endothelial phenotype, 7.8 ± 5.5 % of CD31+ cells (n = 51) and 5.3 ± 3.6 % of CD144+ cells (n = 29) were identified among viable ovarian cells. Vimentin marker is found in 25.6 ± 10.8 % of viable ovarian cells (n = 23) and CD326 (EpCAM expression) in 0.6 ± 0.8 % (n = 16). Finally, pericyte phenotype (CD34-/Vimentin-/CD31-/CD146+/ CD140b+) was identified in 4.6 ± 4.3 % of viable ovarian cells (n = 7).
Limitations, reasons for caution: We do not know how these ovarian cell subpopulations could be a factor associated or not with time for ovarian function recovery *in vivo* after ovarian tissue graft and the impact of these ovarian cells on the ovarian microenvironment of an artificial ovary.
Wider implications of the findings: Functional qualification of ovarian tissue can be performed by MFC. MFC is a promising tool for ovarian cortex qualification before reuse of cryopreserved ovarian tissue. Cell sorting could be used to separate and isolate cell subpopulations and add these cells with isolated follicles in an ovarian reconstruction program.
Trial registration number: not applicable

P-437 The ovaries of transgender men indicate effects of high dose testosterone on the primordial and early growing follicle pool

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Study question: Does high-dose testosterone therapy affect the stage distribution, morphological health and DNA damage repair capacity of human ovarian follicles and their survival *in vitro*?

Summary answer: Testosterone exposure is associated with reduced follicle growth activation, reduced follicle health and increased DNA damage: these further deteriorate after six days of culture. What is known already: Androgens have diverse actions within the ovary, however, there is a lack of information regarding the long-term effects of high-dose testosterone on ovarian function and reproductive potential. Cumulus-oocyte complexes recovered from transgender men have been successfully matured *in-vitro* but little is known regarding the impact of this gender affirming endocrine therapy on the primordial follicle pool. Study design, size, duration: Whole ovaries were obtained from four transgender men aged 25-36 years with informed consent at oophorectomy. All patients had received 1000mg testosterone undecanoate intramuscularly at 12-16 week intervals for a minimum of 4 years pre-operatively. Cortical tissues were dissected into small pieces (≈1x1x0.5mm) and either immediately fixed for histological analysis or cultured for 6 days. Testosterone-treated ovaries were compared to cortical biopsies from age-matched healthy women obtained at caesarean section (n=4, age 26-36). Participants/materials, setting, methods: Follicle number, classification of developmental stage and morphology were evaluated by histological analysis of ovarian cortical tissue from day 0 and 6 days post culture. Immunohistochemical analysis included γH2AX as a marker of DNA damage, and meiotic recombination 11 (MRE11), ataxia telangiectasia mutated (ATM) and Rad51 as DNA repair proteins. A total of 3802 follicles from testosterone exposed and 878 from control ovaries were analysed. Main results and the role of chance: At day 0 (D0), transgender tissue had a higher proportion of non-growing follicles (92.7±1.7%) compared to control (85.4±6.2%, $p<0.05$) but a lower proportion of morphologically healthy follicles (non-growing 59%, primary 61%, secondary 36%; vs 83%, 75%, 80% in controls, all $p<0.005$). After 6 days in culture, the proportion of growing follicles increased (51.3% vs 46.5%) but follicle health further declined (all stages $p<0.005$).

DNA damage was assessed by expression of H2AX. At D0, the proportion of oocytes showing DNA damage was significantly higher in transgender non-growing follicles (48.1±12.5%, vs 12.3±0.25%, $p<0.005$). After culture, H2AX expression increased in both transgender ($p<0.005$) and controls ($p<0.005$) but remained higher in transgender oocytes (non-growing 72.2%, primary 71.7% vs 27.3%, 46.2%, all $p<0.05$). At D0, there was no difference in expression of DNA repair enzymes ATM and RAD51 between transgender and control oocytes, and increased expression of MRE11 in control non-growing follicles ($p<0.05$). Post-culture, there was a significant increase in ATM expression in transgender non-growing oocytes compared to control (98.5% vs 77.8%, $p<0.05$) and a less marked decline in RAD51 expression($p<0.05$). The expression of MRE-11 in control non-growing oocytes dramatically declined (100% to 58.2%, $p<0.05$), unlike in transgender tissue where expression was comparable to D0.

Limitations, reasons for caution: A large number of follicles have been analysed, but only from a small number of ovaries. DNA damage at D0 and after 6 days of culture may not reflect DNA damage and repair capacity at later stages of follicle growth. The effect of duration of testosterone treatment was not investigated.

Wider implications of the findings: These data indicate that high circulating concentrations of testosterone have previously unrecognised effects on the primordial and small-growing follicles of the ovary. These results may have implications for transgender men receiving gender-affirming therapy prior to considering pregnancy or fertility preservation measures.

Trial registration number: n/a

P-438 Effects of prior testosterone use on ovarian stimulation outcomes in transgender men

Abstract withdrawn by the authors

P-439 Carbohydrate metabolism profile during oocyte final maturation reveals culture induced aberrations in in vitro grown and matured mouse antral follicles

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Study question: Are there significant differences in carbohydrate metabolism trends between *in vivo* and *in vitro* grown mouse antral follicles during oocyte final maturation?

Summary answer: Glucose metabolism characterization during GV to MII transition revealed altered metabolic patterns mainly in cumulus cells of *in vitro* grown and matured mouse antral follicles.

What is known already: For some cancer patients fertility restoration is dependent on using efficient *in vitro* follicle culture systems. As human donor ovarian tissue available for research is limited, establishing such culture systems relies on data generated from animal models. The culture system previously developed in our laboratory supports *in vitro* growth of mouse preantral follicles with good oocyte maturation rates but lower developmental competence compared to *in vivo* grown oocytes. Tracking and comparing the metabolic changes after meiotic maturation in *in vitro* and *in vivo* follicles could serve as a screening tool for improving culture conditions and identifying metabolic quality markers.

Study design, size, duration: Mouse secondary follicle culture was performed. *In vitro* grown oocytes, their corresponding cumulus (CC) and granulosa cells (GC) were collected from antral follicles, at germinal vesicle stage (GV) on day 9, and at metaphase 2 (MII) on day 10, after hCG/EGF stimulation. *In vivo* age-matched controls were obtained after intraperitoneal injections with eCG for GV, or with eCG and hCG for MII. *In vivo* GC after ovulation were not included.

Participants/materials, setting, methods: Glucose metabolism trends were compared during final maturation between in vitro grown antral follicles and their *in vivo* controls. Follicles that failed to resume meiosis in vitro were also included.

Enzymatic spectrophotometric assays were used to measure glycolysis, pentose phosphate pathway (PPP), tricarboxylic acid (TCA) cycle, and the antioxidant capacity in individual cell types. Pools of 5 oocytes and corresponding somatic cells were collected, from 3 independent experiments. Unpaired *t*-test was performed with significance when $p<0.05$.

Main results and the role of chance: Important differences were detected between *in vivo* and *in vitro* conditions. GV to MII transition in *in vivo* follicles leads to a metabolic boost in CC as indicated by: i. significant increase in glycolysis, PPP and TCA cycle activity; ii. higher total antioxidant capacity (TAC) ($p<0.05$) and small molecule antioxidant capacity (SMAC) ($p<0.01$). After ovulation, the only significant change in oocytes was an increase in nicotinamide adenine dinucleotide phosphate (NADP+) level ($p<0.01$), possibly due to increased reduced-NADP recycling.

Meiotic maturation triggered no significant differences in any of the metabolic pathways for *in vitro* grown oocytes. Contrary to their *in vivo* controls, in vitro CC showed significant upregulations limited to aconitase, lactate dehydrogenase (LDH) and glutathione-s-transferase (GST) activity ($p< 0.05$). *In vitro* GC showed increased G6PDH activity ($p<0.05$), suggesting PPP upregulation.

Significant differences were detected between *in vivo* GV follicles and the *in vitro* failed-to-mature ones. Oocytes from impaired follicles have higher NADP+ levels ($p<0.0001$) than their *in vivo* immature counterparts. CC showed higher phosphofructokinase (PFK), LDH, catalase activity and increased NADP+ ($p<0.01$), TAC and SMAC ($p<0.05$) compared to *in vivo* GV CCs. GCs from failed-to-mature follicles have significantly higher LDH and superoxide dismutase (SOD) activity than *in vivo* GV GC ($p<0.05$).

Limitations, reasons for caution: The altered metabolic patterns described here in *in vitro* follicles during oocyte GV to MII transition are probably the cumulative effects of both growth and maturation *in vitro*.

Wider implications of the findings: We explored extensively and directly, for the first time, several enzymes and metabolites involved in follicle glucose and redox metabolism in different cell types separately. Understanding of the follicle metabolic requirements is essential for the optimization of follicle culture systems and could lead to development of oocyte quality markers.

Trial registration number: not applicable

P-440 Seven years' experience using oocyte vitrification/warming from in vitro maturation or controlled ovarian hyperstimulation cycles to preserve fertility for oncologic indications

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Study question: Do oocytes vitrified following in vitro maturation (IVM) or controlled ovarian hyperstimulation (COH) for oncologic fertility preservation (FP), lead to similar biological/clinical outcomes after thawing?

Summary answer: IVM is a valid option when chemotherapy is urgent or COH is contraindicated. We report the second live-birth worldwide after IVM in a cancer patient.

What is known already: FP aims at maintaining in cancer survivors, the possibility of childbearing using their own gametes. Currently, oocyte vitrification after COH remains the gold standard but IVM has recently emerged as an option for young women seeking FP, when COH is contraindicated or when cancer therapy is urgent. However, the actual competence of oocyte vitrified after IVM in cancer patients is not established. To date, only one live birth has been reported following frozen/warmed oocytes from an IVM cycle and no data is available comparing biological/clinical outcomes of warmed oocytes resulting either from IVM or COH cycles in cancer survivors.

Study design, size, duration: This retrospective cohort study from a single IVF unit aimed to analyze outcomes of all oocyte warming cycles in 38 cancer survivors having undergone oocyte vitrification for FP after COH or IVM. All of them had oocyte retrieval before administration of gonadotoxic treatment and returned after being cured for assisted reproduction treatments with their oncologist agreement, between January 2014 and December 2020.

Participants/materials, setting, methods: Thirty-eight oocytes warming cycles followed by ICSI respectively from 18 COH and 22 IVM cycles were analyzed. Survival, degeneration following ICSI, fertilization, top-quality and good-quality embryos, defined at day-2 respectively as 4 and 3-5 adequate-sized blastomeres, without multinucleation and containing <20% of cytoplasmic fragments, implantation, biochemical (hCG>100 UI/mL), clinical (intrauterine sac with fetal heart beat) and live birth rates were compared between IVM and COH cycles using appropriate statistical tests. Significance was set at 5%.

Main results and the role of chance: The indications for FP were breast cancer (n=32), hematologic malignancies (n=3), BRCA1 mutation (n=2), borderline ovarian tumor (n=1). The mean age and antral follicle count (AFC) at the time of FP was similar in both groups. The number of cryopreserved oocytes was significantly lower in the IVM group (5.7 ± 9.1) when compared with the COH group (11.4 ± 3.3; p=0.009). Oocyte survival rates were similar in IVM (70 ± 24%) and COH groups (73 ± 28%). Although not significant, we reported a trend to better results in the COH group when compared with those of IVM group in terms of degeneration rate following ICSI (6 ± 10% vs. 14 ± 20%; p=0.16), fertilization (72 ± 35% vs. 54 ± 27%; p = 0.08), day 2 top-quality (38 ± 32% vs. 21 ± 31%; p=0.15) and good-quality embryo (46 ± 30% vs. 25 ± 30%; p=0.06), implantation (18 ± 35% vs. 14 ± 36%; p=0.79), biochemical (28 (5/18) vs. 14% (3/22); p=0.26), clinical (22 % (4/18) vs. 9% (2/22); p=0.24), live birth rates (22 % (4/18) vs. 5% (1/22); p=0.06).

Limitations, reasons for caution: Caution is needed when interpreting these retrospective data obtained from a limited number of frozen-thawed cycles. Statistical power to compare IVF outcomes after COH and IVM is limited by the few women who return for oocyte reutilization.

Wider implications of the findings: The present investigation is the largest evaluating the IVM-oocyte frozen-thawed cycles in a oncologic population. It suggests that a higher oocyte yield may be necessary in IVM, since fertilization/embryo-quality rates seem lower. Success rates and limiting factors of oocyte vitrification in this context is needed for providing proper oncofertility counseling.

Trial registration number: not applicable

P-441 Semen quality and cryopreservation in adolescent transgender females

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Study question: What are the semen quality and cryopreservation outcomes among adolescent transgender females at the time of fertility preservation (FP) before initiating gender-affirming hormone (GAH) treatment?

Summary answer: Semen quality is strongly reduced among adolescent transgender females before hormone therapy and their stored sperm samples are suitable for intracytoplasmic sperm injection (ICSI).
What is known already: The age of individuals seeking treatment for gender affirmation has fallen sharply in recent years and many of them are adolescents. Estrogen, the primary treatment for transgender women, is known to impair semen quality and fertility potential. Sperm cryopreservation enables young transgender females to circumvent GAH therapy-related fertility impairment and have genetically related children. There are recent data on semen quality among adult transgender women who preserve fertility before exposure to GAH therapy, but little is known about pubertal transgender female adolescents.
Study design, size, duration: This retrospective cohort study included 26 adolescent transgender females who underwent FP between June 2013 and October 2020.
Participants/materials, setting, methods: Before initiating gonadotropin-releasing hormone agonists solely or with GAH treatment, 25 adolescent transgender females were referred to FP in our Fertility Institute of a tertiary university-affiliated medical center. Pre-freezing semen parameters were compared to WHO 2010 reference values. Post-thaw semen parameters were used to determine adequate assisted reproductive technology (ART). A multivariate linear regression analysis was performed to assess the impact of selected medical and lifestyle factors on the semen quality of our study participants.
Main results and the role of chance: The mean age at which adolescent transgender females underwent sperm cryopreservation was 16.2 ± 1.38 years. The median values of all semen parameters in our study group were significantly lower compared to the WHO data on semen quality in the general population of unscreened men, including volume (1.46 ml vs 3.2 ml, respectively, $P = 0.001$), sperm concertation (28*106/ml vs 64*106/ml, $P < 0.001$), total sperm number (28.2*106 vs 196*106, $P < 0.001$), total motility (51.6% vs 62%, $P < 0.001$), and normal morphology (2% vs 14%, $P < 0.001$). The frequency of semen abnormalities was teratozoospermia 72%, hypospermia 52%, oligozoospermia 28%, and azoospermia 4%. The median post-thaw total motile count was 0.17*106 per vial, and the quality was adequate only for ICSI in 87.7% of the thawed semen samples. Attention-deficit/hyperactivity disorder (ADHD) diagnosis, history of depression/anxiety, medication for ADHD, and antidepressant drugs were found to correlate with hypospermia. No correlation was found between the time of FP, body mass index, autistic spectrum disorder diagnosis, cannabis use, testis tucking, or the levels of follicle-stimulating hormone, estradiol, and testosterone on the semen parameters.
Limitations, reasons for caution: Because no normal values of semen in adolescents are available and the absence of a matched control group, we used WHO 2010 semen data as reference values, and they may not be representative of the adolescent population.
Wider implications of the findings: Although adolescent transgender females have poor semen quality and limited stored semen samples suitable for advanced ART interventions, even before starting GAH therapy, we highly recommend sperm cryopreservation before initiating GAH treatment and thereby prevent further impairment of sperm quality associated with the hormonal treatment.
Trial registration number: Not applicable

P-442 Cancer does not adversely affect oocyte maturation in vitro with the exception of breast cancer

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Study question: Do different types of cancer affect the success of oocyte maturation *in vitro* compared to infertile women included in the *in vitro* fertilization (IVF) program?
Summary answer: Cancer does not adversely affect oocyte maturation *in vitro*, with the exception of breast cancer, compared to infertile women in the *in vitro* fertilization program.
What is known already: Vitrification and storage of oocytes in liquid nitrogen is one of the real options for maintaining reproductive function in cancer patients. Despite careful hormonal stimulation of the ovaries, however, the proportion of oocytes is immature and lost to the patient. *In vitro* maturation of oocytes can play an important role in resolving immature oocytes and increasing the chances of conception in cancer patients. Moreover, it can mean a safe way to store oocytes when ovarian hormonal stimulation could worsen the disease. Therefore, the aim of this study was to determine whether different types of cancer affect oocyte *in vitro* maturation.
Study design, size, duration: After ovarian stimulation in 18 cancer patients, the number and maturity of oocytes were compared to 21 infertile patients in the IVF program over a three-year period. In both groups, 119 germinal vesicle-GV oocytes were matured *in vitro* to compare the maturation rate. After IVF in a subset of 17 infertile patients, the fertilization of *in vitro* and *in vivo* matured oocytes was compared in the same cycles. The procedure was considered in cancer patients.
Participants/materials, setting, methods: In this prospective study, forty-five GV oocytes in cancer patients and 74 GV oocytes in infertile patients underwent *in vitro* maturation procedure. Each oocyte was matured *in vitro* in the MediCult IVM System by conditioning in LAG medium and maturation for up to 28 hours in IVM medium with added hormones FSH and hCG, in coculture with cumulus cells from mature oocytes in the same patients. Oocytes were fertilized by intracytoplasmic sperm injection (ICSI).
Main results and the role of chance: After controlled ovarian hormonal stimulation, 198 oocytes were retrieved in cancer patients and 259 oocytes in infertile women and there were no significant differences in the number of retrieved oocytes, proportion of degenerated oocytes and proportion of GV oocytes. In cancer patients, the proportion of oocytes that matured *in vitro* was lower than in infertile patients (66.0 vs. 80.0%), but the difference was not significant. Among cancer patients, the oocyte maturation rate tended to be lower in patients with breast cancer than in patients with other cancers (54.5% vs. 81.2%; difference not significant). However, in patients with breast cancer, significantly fewer oocytes matured *in vitro* than in infertile patients (54.5% vs. 80.0%; P < 0.05, Chi-Square test) even though they tended to be younger (29.3 ± 7.4 vs. 33.4 ± 5.0 years; non-significant difference). After *in vitro* maturation, there was a 13% increase in mature oocyte yield in cancer patients and a 20.1% increase in infertile women with no significant difference observed. After ICSI in a subset of infertile women, there was approximately the same fertilization rate between oocytes matured *in vitro* and *in vivo* (55.1% vs. 57.0%) in the same cycles.
Limitations, reasons for caution: For ICSI in oocytes matured *in vitro*, we had to use semen collected the day before, while oocytes matured *in vivo* were fertilized with fresh semen in the same cycle. Therefore, we could not compare the development of embryos in both groups.
Wider implications of the findings: *In vitro* maturation of oocytes in connection with their vitrification or vitrification of embryos after their fertilization appears to be a valuable way to maintain the fertility of young cancer patients, but a worse outcome is expected in breast cancer patients.
Trial registration number: National Medical Ethical Committee Approval, No. 0120-222/2016-2; KME 115/04/16.

P-443 Effects of capsaicin pre-treatment on ovarian follicle pool, inflammatory and apoptotic pathways against radiation induced ovarian failure

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Study question: Is capsaicin effective in preventing radiation induced ovarian follicle loss and premature ovarian failure (POF) in rats?
Summary answer: Capsaicin pre-treatment before radiotherapy restores especially primordial follicle pool, inhibits atresia of ovarian follicles, may be an acceptable therapeutic modality to prevent radiation induced POF.
What is known already: Ionizing radiation exposure to pelvic area induces inflammation, oxidative stress, follicular atresia and apoptosis; leading to POF. Phytochemicals were used in animal studies to prevent radiotherapy induced POF because of their antioxidant and anti-inflammatory properties however their potential radio-protective effects in human ovarian follicles are not clear. Capsaicin is the active compound of hot peppers and has anti-inflammatory and

antioxidant properties. It was found that low dose capsaicin stimulated ovarian follicular development and proliferation of granulosa cells, inhibited apoptosis of ovarian follicles in pre-pubertal rat ovaries. However, no data exists on radio-protective effects of capsaicin on ovarian follicles.

Study design, size, duration: Twenty-four young adult Wistar albino female rats were housed under standard conditions (20 ± 1 0C room temperature, 60 ± 10 % humidity, and a 12/12-h light/dark cycle) in regular cages and allowed free access to food and water. After 10 days of subcutaneous capsaicin 0,5 mg/kg/day or placebo treatment, animals exposed to total body irradiation of 8.3 Gy using a linear accelerator. Treatment continued for 1 day after irradiation.

Participants/materials, setting, methods: Rats were randomly divided into four groups: (1) control: non-irradiated rats were injected placebo; (2) capsaicin: non-irradiated rats were injected capsaicin; (3) radiation only (IR): rats were injected placebo before exposure to a single dose of 8.3-Gy whole body radiation; (4) Radiation-capsaicin (IR+CAP): rats were injected capsaicin prior to whole body irradiation and continued for 1 day after irradiation. Rats were sacrificed, blood samples were obtained for biochemical investigations. Ovaries were dissected for histopathological evaluation.

Main results and the role of chance: Radiation triggered oxidative stress, increased ovarian inflammation, increased follicular apoptosis and diminished ovarian follicle pool. Capsaicin was significantly ameliorated; oxidative stress by decreasing serum total oxidant status, oxidative stress index, disulfide, and malondialdehyde levels ($p \leq 0.001$ both); ovarian inflammatory status by decreasing expressions of TNF-α, IL-1β, poly ADP-ribose polymerase-1 (PARP-1) ($p=0.002$ both); apoptosis by decreasing expressions of active caspase-3 and p53 ($p=0.015$ and $p=0.002$ respectively); follicle counts by increasing primordial follicles and decreasing apoptotic follicles ($p \leq 0.001$ both) in rats when administered before radiation exposure. Results of our study confirmed previously reported pro-proliferative and anti-apoptotic properties of capsaicin on ovarian follicles. These beneficial effects of capsaicin are demonstrated for the first time on ionizing radiation exposed rat ovaries.

Limitations, reasons for caution: Present study is a in-vivo rat study and other preclinical studies are needed to confirm our findings before moving forward to human trials. Radio-protective effects of capsaicin on rat ovarian follicles were demonstrated only in short term. Long term effects of capsaicin on folliculogenesis, fertilization and fecundity should be investigated.

Wider implications of the findings: Preserving fertility is one of the main goals of successful radiotherapy in terms of quality of life for oncological or hematological diseases. Capsaicin treatment before radiotherapy may be an acceptable therapeutic modality to prevent radiation induced POF and has potential to utilize in clinical application in terms of fertility preservation.

Trial registration number: 218S876/2019

P-444 Presence of pharmacological inhibitors of the PI3K/PTEN/Akt and mTOR signalling pathways during cryopreservation and organotypic cultures of murine ovaries limits early primordial follicle depletion

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Study question: Which signalling pathways are implicated in primordial follicle activation induced by cryopreservation and/or organotypic culture? Is it possible to limit this activation through pharmacological inhibitors?

Summary answer: Our findings provide support for the hypothesis that mTOR and PI3K inhibitors might represent an attractive tool to delay cryopreservation- and culture-induced primordial follicle activation.

What is known already: Cryopreservation of ovarian tissue containing immature primordial follicles followed by auto-transplantation (OTCTP) is the only option available to preserve the fertility of prepubertal patients or patients requiring urgent therapy for aggressive malignancies. However, a major obstacle in this process is follicular loss immediately after grafting, possibly due to slow neovascularization, apoptosis and/or massive follicular recruitment. *In vitro* and *in vivo* studies indicate that the PI3K/PTEN/Akt and mTOR signalling pathways are involved in follicle activation. The transplantation process seems to be the major cause of primordial follicle activation after OTCTP but information about how cryopreservation itself impacts follicle activation is sparse.

Study design, size, duration: Whole murine ovaries (4-8-weeks old) were cryopreserved by slow freezing and exposed to LY294002 (a powerful PI3K inhibitor) or rapamycin (a specific mTOR inhibitor) during cryopreservation and/or organotypic *in vitro* culture for a 24 h or 2 days.

Participants/materials, setting, methods: Western Blot and immunofluorescence analyses were used to determine the activation of PI3K/PTEN/Akt and mTOR signalling pathways in murine ovaries cryopreserved and/or organotypically cultured with/without inhibitors.Follicles were quantified according to their maturation degree on H&E stained histological sections.

Main results and the role of chance: Ratio of phosphorylated Akt or rps6 to total proteins (p-Akt/Akt and p-rps6/rps6) was increased in slow-frozen murine ovaries compared to control fresh ovaries, indicating an activation of the PI3K/PTEN/Akt and mTOR signalling pathways. The use of pharmacological inhibitors of follicle signalling pathways (LY294002 (25μM) and rapamycin (1μM)) during the cryopreservation process decreased p-Akt/Akt and p-rps6/rps6 ratios. *In vitro* organotypic culture for 24 h increased only the activation of the PI3K/PTEN/Akt pathway, as shown by increased p-Akt/Akt ratio in fresh ovaries cultured for 24 h compared to fresh non-cultured ovaries. This activation can be counteracted by cryopreservation of murine ovaries with rapamycin followed by *in vitro* culture for 24 h in the presence of LY294002. Follicle density quantifications indicated that when cryopreserved ovaries were maintained in culture for 2 days, a decrease of primordial follicle density concomitant with an increase of secondary and more mature follicles were found in comparison to slow-frozen/thawed ovaries without culture. Supplementation of the culture medium with LY294002 and rapamycin for 24 h or 2 days preserved primordial follicle densities compared to ovaries cultured without inhibitors.

Limitations, reasons for caution: This study is an *in vitro* study using murine ovaries. To analyze the efficiency of LY294002 and rapamycin to limit cryopreservation and transplantation induced follicle recruitment, these inhibitors should be tested in an *in vivo* model. Furthermore, these findings will need to be confirmed with human samples.

Wider implications of the findings: We showed for the first-time that the sequential use of pharmacological inhibitors, rapamycin during the slow freezing process followed by organotypic culture supplemented with LY294002, is effective to limit early primordial follicle depletion.

Trial registration number: /

P-445 Successful live birth after repeated high-dose radiotherapy to the uterus

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Study question: It has been established that radiotherapy can increase the risk of adverse pregnancy outcomes. However, there is currently no consensus on the effective sterilizing dose for adulthood uterine radiotherapy.

Summary answer: Uterine fertility preservation methods should be guided by the age of the patient receiving radiotherapy and the actual dose of radiation exposure to the uterus.

What is known already: Many experts have suggested that a high dose of radiation to the uterus is a reason to counsel patients against future pregnancy. There are major limitations to the current literature regarding off-target radiation damage to the uterus. One study reported a relative risk of 9.1 for stillbirth and neonatal death after 10 Gy doses.

Study design, size, duration: Case report and review of the literature before December 2020

Participants/materials, setting, methods: A case report of a 36-year-old female with three cancers and received repeated high-dose radiotherapy of 66 Gy and 50 Gy to the pelvis. We used a dose-volume histogram, the most widely used tool to calculate the radiation distribution within a volume of interest of the

patient during radiotherapy. We determined that her uterus may have received the highest uterine radiation dosage for full-term live birth in current literature.
Main results and the role of chance: Due to iatrogenic ovarian failure, she could only use donor eggs. After endometrium preparation for 18 days, the endometrium reached 8.7 mm with a triple-line appearance. We transferred two cleavage-staged embryos and one of them implanted successfully. The course of the pregnancy was uneventful. Finally, the patient gave birth to a healthy baby via Cesarean section at 38 5/7 weeks of gestation.
Limitations, reasons for caution: It should be noted that the success of our case may not apply to all patients with cancer after they have received RT. We should inform patients about the increased risk of preterm birth, low birth weight infants, uterine rupture, and neonatal death.
Wider implications of the findings: The patient's age and the dose of RT exposure to the uterus are important factors for the prognosis of a future pregnancy. More well-designed studies will be needed to allow future standard guidelines for uterine fertility preservation.
Trial registration number: TMU-JIRB N20204149

P-446 Controlled ovarian stimulation protocols for oocyte vitrification induce differential gene expression profiles in primary tumours of breast cancer

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Study question: Could controlled ovarian stimulation (COS) protocols used in fertility preservation (FP) impact on malignant cell proliferation and tumour molecular profiling of breast cancer (BC) patients?
Summary answer: Letrozole supplementation during ovarian stimulation for oocyte vitrification could be considered as a safe procedure in estrogen-dependent BC patients undergoing FP.
What is known already: High estradiol levels associated to COS could promote changes in gene expression in estrogen-positive BC tumors. Estradiol levels reached during the ovarian stimulation could aggressively promote malignant cell proliferation and cell migration to adjacent organs. Aromatase inhibitors such as letrozole, are added to standard stimulation protocols to avoid this undesirable potential side effect. Despite the reassuring clinical results achieved by using letrozole for FP in BC patients, there is still a lack of evidence regarding its impact on malignant cell behaviour. For this reason, specific molecular studies to properly evaluate safety of letrozole in this specific population are still required.
Study design, size, duration: Experimental in vivo study. Thirty 5-week-old Nude-nu female mice were divided into three different groups: BC (n=10), BC and FSH stimulation (BC-FSH, n=10), or BC and letrozole stimulation (BC-LTZ, n=10). BC was considered the control group, whereas BC-FSH and BC-LTZ represented distinct COS protocols. Hormone-dependent BC was induced in all mice. Animals were followed-up for 5 months and then euthanized to collect kidney, ovary, spleen, and liver tissues for gene expression and immunohistochemistry (IHC) analysis.
Participants/materials, setting, methods: One million of human MCF-7 BC cells were injected into the mouse left kidney capsule. Two days after xenograft, COS was induced by 10IU FSH or 1mg/ml letrozole + 10IU FSH, followed by ovarian triggering with 10IU hCG at 48h. Human BC RT2 Profiler PCR Arrays were performed to evaluate the impact of COS on tumour behaviour. BC biomarkers (Ki67, Er , PR and HER-2) were also analyzed by IHC to validate gene expression results.
Main results and the role of chance: The differential gene expression was firstly assessed in kidney samples, as they represent the xenograft site, and different expression profiles were obtained depending on the COS protocol used. The BC-FSH group showed a global over-expression pattern of all genes of the array when compared to BC and BC-LTZ. Further gene ontology analysis revealed that cellular process, biological regulation, metabolic process, and proteases were the most over-represented biological terms, with a 20.5-fold over-expression for MMP2 compared to the other groups. On the other hand, BC-LTZ mice presented gene expression profiles similar to that of controls. When other tissues were analysed to detect malignant cell presence, our results revealed a significant up-regulation of matrix-proteases, cell cycle and proliferation related-genes, in liver samples from the BC-FSH group, but no amplification of any of the studied genes was detected in ovarian tissue or spleen. IHC findings confirmed the presence of human BC cells in 100% of samples from kidney tissue and in 30% of samples from liver tissue in the BC-FSH group. No human cells were detected by IHC in the BC and BC-LTZ groups.
Limitations, reasons for caution: Since this is an animal model of estrogen-dependent BC induced through a cell line, further validation with human tumour breast cancer samples would be required.
Wider implications of the findings: Adjuvant letrozole in COS protocols prevents BC cell migration. The present study suggests that this protective effect could be mediated by interfering ER-pathway downstream genes involved in cell proliferation and matrix digestion. Altogether, letrozole could safely be used as a supplement during COS procedures for oocyte vitrification in BC women.
Trial registration number: Not applicable

P-447 Challenging cases in Oncofertility: Insights from a national specialized e-meeting for fertility preservation specialists

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Study question: How a new specialized e-meeting for complex cases in oncofertility is used by fertility preservation specialists (FPS)?
Summary answer: The e-meeting for complex oncofertility cases is an innovative tool that fulfils the needs of FPS and could help them to improve their oncofertility practice.
What is known already: Little is known about the management of fertility preservation (FP) in rare cancer that could be challenging for FPS due to lack of experience and scientific data. To our knowledge, there is no specially dedicated meeting reported in published literature to provide highly specialized advices to FPS in these challenging situations of FP.
Study design, size, duration: We present three years of activity of a national French e-meeting dedicated to the management of challenging oncofertility cases. We conducted a retrospective analysis of all submitted cases. Second, a survey was conducted to evaluate the use of this e-meeting at participating FPS.
Participants/materials, setting, methods: The E-meeting for Complex Cases in Oncofertility was created in 2016 September in France, allowing for National oncofertility experts to share viewpoints about challenging cases for which they do not have experience and/or no sufficient data available in published literature. Demographic and clinical data, number of replies and proposal for each case were collected retrospectively. A survey to investigate the use and the interest of FPS for this tool was sent to its members. Main results and the role of chance: One hundred and four experts have joined the e-meeting since its set-up and 109 challenging cases have been submitted. Mean age of patients was 22.0 ± 8.9 years old and 87% were female. Most of cases were hematological cancers (n=32/109; 29%), gynecologic cancers (n=30/109; 27%) and neurological cancers (n=12/109; 10.9%). Each submitted case received on average 2 ± 1 different strategy for FP and the opinion of 7 ± 2 experts. Among the FPS who submitted cases, seeking the opinions from others FPS allowed them to confirm their care plan (N=49; 84%), to offer different options to their patient (N=34; 58%) and to compare

their practices with other specialists (N=23; 39%). All respondents reported a self-perceived improvement in their practice of oncologic FP (n=80; 100%).
Limitations, reasons for caution: Although this study showed a perceived improvement at FPS in the management of challenging oncofertility cases, we did not study in details their adherence to the e-meeting's proposals. The value of this new tool has also not been assessed regarding patient's quality of life and further fertility.
Wider implications of the findings: Specific attention should be paid for challenging cases in oncofertility for which only experiences of individual exist. Enhancing communication between FPS through national and international networks, pooling experiences and collecting the most complex cases are required in order to improve the management of these patients.
Trial registration number: Not Applicable

P-448 Clinical outcome of social oocyte cryopreservation at advanced age

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Study question: What are the success rates of social oocyte cryopreservation (SOC) at advanced age?
Summary answer: In this study, one in four women who underwent SOC above age 35 had a delivery.
What is known already: While SOC is gaining popularity, reports on delivery rates are limited due to low utilization rates.
Study design, size, duration: Retrospective data collection of all woman who underwent SOC between 2011-2018, and presented for treatment using cryopreserved oocytes until January 2021. Participants/materials, setting, methods: Review of patient records (including both IVF and antenatal/postnatal) and laboratory data in a university affiliated hospital-based IVF unit. Main results and the role of chance: A total of 448 women underwent SOC during 2011–2018. 50 (11.2%) women returned to use these oocytes until the end of January 2021. Women who returned to use their oocytes underwent cryopreservation at mean age of 38.2±2.2. 46 (92%) of participants were above 35 at time of cryopreservation. Number of oocytes cryopreserved was 11.3±9.7. Mean time from cryopreservation to thawing was 5.5±1.8years (range 1-9 years). and age at thawing was 43.4±2.1 (range 40-49). Nearly half of patients initially attempted to conceive before using their cryopreserved oocytes, mostly by ART using fresh oocytes. Mean number of oocytes thawed and oocytes survived per women was 9.7±6.2 and 6.1±4.9 respectively (post thawing survival rate 65.4±35%).

Mean number of embryos transferred, at one or more attempts was 2.6±2.1 per women. Eleven women gave birth or had an ongoing pregnancy > 20 weeks at time of analysis. All deliveries resulted from cryopreservation at age 36 and older (delivery rate 23.9% per women). Limitations, reasons for caution: We report our initial experience of women who underwent SOC at a single center. Most women who returned to use their oocytes had undergone SOC at advanced age, therefore not necessarily reflecting outcome for younger patients attempting to preserve fertility using this technology. Wider implications of the findings: Considering modest success rates of SOC in our cohort, women considering SOC are advised to do so at an earlier age.
Trial registration number: not applicable

P-449 Evaluation of ovarian reserve in female children and adolescents with non-iatrogenic primary ovarian insufficiency to establish criteria for ovarian tissue cryopreservation

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Study question: What is the predictive value of ovarian reserve evaluation in patients with non-iatrogenic primary ovarian insufficiency (NIPOI) for follicle detection in ovarian tissue harvested for cryopreservation?
Summary answer: Ovarian tissue cryopreservation (OTCP) should be considered if patients present at least one of the following parameters: detectable AMH, FSH≤20mIU/ml, detection of ≥1 antral follicle.
What is known already: In pre-pubertal girls suffering from NIPOI, which majorly has a genetic etiology, fertility preservation using OTCP is commonly practiced. When OTCP was performed in an unselected group of children and adolescents with NIPOI, only 26% of them had follicles in ovarian tissue while 74% did not benefit from the surgery. The role of preoperative evaluation of anti-müllerian hormone (AMH) serum level, follicular stimulating hormone (FSH) serum level, and trans-abdominal ultrasound for the antral follicle count to predict the detection of primordial follicles in the harvested ovarian tissue is unclear.
Study design, size, duration: We conducted a retrospective analysis of all patients ≤ 18 years old who were referred for fertility preservation counseling due to NIPOI at a single tertiary hospital between 2010 and 2020. If initial evaluation suggested a diminished ovarian reserve and at least one positive parameter indicating a follicular activity (AMH > 0.16ng/ml, FSH ≤ 20mIU/ml, detection of ≥ 1 antral follicle by transabdominal sonography), OTCP was offered. Patients with 46XY gonadal dysgenesis were excluded.
Participants/materials, setting, methods: OTCP was performed laparoscopically in all cases. A fresh sample of cortical tissue was fixed in buffered formaldehyde for histological analysis. The rest of the ovarian tissue was cut into small cuboidal slices 1-2 mm in thickness and cryopreserved. After the serial sections, the histological slides were evaluated for the presence of follicles by a certified pathologist. Follicles were counted and categorized as primordial, primary, and secondary.
Main results and the role of chance: During the study period, 39 patients with suspected NIPOI were referred to the fertility preservation center. Thirty-seven patients included in the study were diagnosed with Turner's syndrome (n=28), Galactosemia (n=3), Blepharophimosis-Ptosis-Epicanthus Inversus syndrome (n=1), and idiopathic NIPOI (n=6). Of 28 patients with Turner's syndrome, 6 had 45X monosomy, 15 had mosaicism and 7 had structural anomalies in X-chromosome. One patient with gonadal dysgenesis and one with the presence of Y-chromosome in 20% of somatic cells were excluded from the study. OTCP was conducted in 14 patients with at least one positive parameter suggesting ovarian function. No complications of the surgical procedure or the anesthesia were observed. Primordial follicles were found in all patients with two or three positive parameters (100%) and in three of six cases with one positive parameter (50%). In total, of the 14 patients who underwent OTCP with at least one positive parameter, 11 (79%) had primordial follicles at biopsy (mean 23.9, range 2-47). This study demonstrates a positive predictive value of 79% for the detection of primordial follicles in patients who had at least one positive parameter of ovarian reserve evaluation. If two or three parameters were positive, the positive predictive value increased to 100%.
Limitations, reasons for caution: This study did not examine the negative predictive value of our protocol as OTCP was not recommended in the absence of positive parameters. The future fertility potential of cryopreserved tissue in the population with NIPOI is unclear and should be discovered in further studies.
Wider implications of the findings: We suggest the evaluation of ovarian reserve by antral follicles count, AMH, and FSH serum levels prior to OTCP in patients with NIPOI. By recommendation of OTCP only if ≥ 1 parameter suggesting the ovarian function is positive, unnecessary procedures can be avoided.
Trial registration number: not applicable

P-450 Effects of rheumatoid arthritis and methotrexate therapy on ovarian reserve in infertile women

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Study question: Do patients with infertility and rheumatoid arthritis (RA) treated with methotrexate (MTX) have ovarian reserve alterations?
Summary answer: Women with infertility and RA treated with MTX were found to have statistically significant decrease of ovarian reserve.
What is known already: Rheumatoid arthritis (RA) is one of the most prominent inflammatory diseases affecting women of child-bearing age [Brouwer J. et al, 2014]. RA and its treatment may interfere with the female reproductive

physiology. The vast majority of patients with RA are treated with methotrexate (MTX) which is a folate antagonist that inhibits DNA synthesis. MTX, which is the anchor drug in RA, targets actively proliferating cells including the oocytes and granulosa cells which may impair the ovarian reserve [Min Tun Kyaw et al, 2020].

Study design, size, duration: A prospective case-control study that enrolled 72 female patients with infertility was conducted in the 2-year time period of September 2018 to October 2020.

Participants/materials, setting, methods: The main group comprised 32 patients with infertility and RA; the control group consisted of 40 women with infertility only. Patients with RA were stratified into subgroups based on whether or not they received MTX.

To investigate ovarian reserve measurement of serum anti-Müllerian hormone (AMH) was used. The level of AMH was evaluated concerning RA duration and activity, as well as the age at initiation of MTX therapy, dosage, and treatment duration.

Main results and the role of chance: The mean age of the study population was 36±3 years. The duration of RA was 4 [3; 11] years. The low disease activity based on DAS28-ESR (disease activity score based on 28 joints using the erythrocyte sedimentation rate) prevailed(56.2%).

In the main group 19(59.4%) women received MTX therapy. The MTX dosage was 15 [15;20]mg /wk, the duration of MTX therapy by the day of inclusion in the study was 18.7[1; 15]months.

The AMH level was significantly lower in the main group (2.1 n /ml vs 2.73ng /ml, p=0.043). The number of patients with decreased ovarian reserve (AMH level<1.0ng/ml) significantly prevailed in the group of patients with RA (25% vs 5%, p=0.015).

When assessing the AMH level in patients with RA who received MTX (n=19) and patients in the control group, there was a tendency towards a decrease in the indicator in the first subgroup, but no statistically difference was found (p=0.074).

Correlation analysis of the dependence of AMH level on the patient age showed the most significant decrease in AMH in the patients with RA receiving MTX compared to the patients with RA who did not, and compared to all patients with RA regardless of the therapy received (rs=-0.563) (p <0.05).

Limitations, reasons for caution: The lack of statistically significant data in certain cases may be due to the small sample size.

Wider implications of the findings: RA and MTX administration are associated with a significant decrease in AMH levels. The age of initiation of the therapy is negatively correlated with the AMH level. In this regard, patients with already compromised reproductive function who are planning to receive MTX should be advised to preserve the genetic material.

Trial registration number: 567890

P-451 Improving neovascularization and follicle viability in cryopreserved bovine ovarian tissue transplants

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Study question: Does cryopreservation and transplantation of bovine ovarian medulla-containing cortex tissue improve the viability and vascularization of the graft? Summary answer: Transplantation of bovine ovarian cortex containing medulla has a positive effect on follicular viability and neovascularization of the graft compared to cortex transplantation alone.

What is known already: For female fertility protection, cryopreservation and retransplantation of ovarian tissue is a widely used method. During cryopreservation, ovarian tissue is exposed to mechanical and hypoxic stress resulting in follicular loss. Moreover, after retransplantation tissue vitality and follicle survival is limited due to ischemia. As follicular viability is of major importance for fertility and hormonal activity, the main focus is on improving vitality and viability of the grafts. In current protocols, ovarian medulla is discarded and merely cortex tissue is preserved. However, medulla tissue predominantly contains blood vessels, thereby obtaining high potential for revascularization processes and thereby supporting tissue vitality.

Study design, size, duration: This experimental laboratory work was performed during a period of ten months. The rapidly vascularized chorioallantoic-membrane (CAM) of fertilized chicken eggs was used as model system to investigate neovascularization, follicle survival and tissue vitality of different bovine ovarian grafts. In four independent experimental rows four different tissue types (isolated cortex, thick medulla-containing cortex (8 x 10 x 3 mm), thin medulla-containing cortex (5 x 10 x 3 mm) and sole medulla tissue were compared.

Participants/materials, setting, methods: Out of four bovine ovaries preserved from the slaughterhouse, in total 117 samples of the four different tissue types were primed and cryopreserved by the common slow-freezing protocol. After thawing, grafts were transplanted on separate CAMs at day four of fertilized eggs. After four days of incubation, blood vessels growing towards the grafts were counted. Subsequently, grafts were harvested, digested with collagenase and stained with Neutral Red® to determine the total amount of vital follicles.

Main results and the role of chance: To investigate the neovascularization, all graft-supplying blood vessels were determined and distinguished between small and thick vessels. Compared to sole cortex, there were more small vessels in the medulla-containing grafts (9,72 vs. 8,65). Especially thin medulla- containing cortex pieces exhibited the highest number of small vessels (9,90). Also in isolated medulla tissue an increased amount of small vessels was observed (9,79). However, the average number of big vessels was not significantly different in all four test groups (Cortex: 2,12; thin medulla-containing cortex: 1,69; thick medulla-containing cortex: 1,5; medulla: 2). The total number of all vessels differed from 10,76 (sole cortex) to 11,75 (medulla-containing grafts), indicating a support of neoangiogenesis by medulla tissue. To further examine whether medulla tissue also alters the amount of vital follicles, Neutral Red® stained vital follicles were determined in all different sample groups. Indeed, in medulla-containing cortex samples was an augmented average number of vital follicles (342,4) compared to sole cortex tissue (256,11). Most vital follicles were detectable in the thick medulla-containing cortex tissue (346,61), closely followed by the thin medulla-containing cortex grafts (338,19). As expected, there was just a rare amount of vital follicles in sole medulla grafts (8,13).

Limitations, reasons for caution: As the ovarian reserve in cattle is very individual, the prepared ovaries are different in their follicle amount. These individual differences may influence the number of counted follicles. Furthermore, the CAM model is only a short term experimental approach to investigate neovascularization and follicle survival.

Wider implications of the findings: According to our results, transplantation of human medulla-containing cortex appears promising. Keeping medulla tissue on the graft seems to improve both follicle viability and revascularization. Our findings need to be proven with human tissue, but might change the preparation of human ovarian tissue for fertility preservation in future.

Trial registration number: not applicable

P-452 The challenge of ovarian tissue culture: 2D versus 3D

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Study question: Does an alginate matrix scaffold improve ovarian tissue culture?
Summary answer: Ovarian tissue culture within an alginate scaffold has no advantage over conventional culture, being more time consuming and less reproductible
What is known already: Cryopreservation of ovarian tissue is a powerful technique for preserving female fertility, as it can restore fertility and endocrine function. Several studies have been carried out aiming to increase the longevity of the transplant and decrease the risk of reimplantation of neoplastic cells. For *in vitro* follicle culture, recent research has shifted from two dimensional (2D) toward the use of three-dimensional (3D) structures. The use of a matrix maintains the architecture and mimics in vivo conditions, with a variable access to oxygen and nutrients. This bridges the gap between conventional cell culture and animal models.
Study design, size, duration: Ovarian tissue fragments were divided into 2 groups: conventional culture (2D culture) and culture using an alginate matrix scaffold (3D culture). Tissue was evaluated at four time-points: immediately after thawing and after 24, 48 and 72 hours of culture.
Participants/materials, setting, methods: Rat ovarian tissue was cryopreserved and thawed with validated protocols. Follicular analysis was conducted after haematoxylin and eosin staining, regarding density, classification and degeneration. Tissue viability was assessed using lactate dehydrogenase (LDH) levels in supernatants and histological score. Three parameters were considered, namely, interstitial oedema, follicular cell degeneration and percentage of tissue in necrosis. Apoptosis was assessed by caspase 3 immunostaining. Proliferating cells were identified using Ki67 immunohistochemical labelling.
Main results and the role of chance: Follicular density, cell proliferation and apoptosis both in follicles and stroma was similar in both culture conditions. Stromal cells proliferation was stable in conventional culture but decreased in 3D culture (p=0.001), which can be explained by the rigidity of alginate matrix. At 24 hours of culture, cytotoxicity was lower in the 3D model (p=0.006), due to low levels of LDH in the supernatant, that may be related to retention within the matrix. As culture time increased cytotoxicity seemed to be similar. Degradation of the tissue was suggested by the histological score analysis of tissue during 72 hours of culture. Tissue injury was greater (p=0.01) in 3D culture due to higher interstitial oedema (p=0.017) and tissue necrosis (p=0.035). In the interior of the alginate scaffold, the bioavailability of oxygen and nutrients may be limited, affecting cell survival over time and conditioning higher level of necrosis and release of intracellular content.
Limitations, reasons for caution: There are two major limitations that should be addressed in future research, namely the study of the tissue-matrix interactions and culture medium supplementation to decrease follicular atresia.
Wider implications of the findings: There is no advantage in the use of an alginate matrix scaffold for ovarian tissue culture, as it is more time consuming, difficult to perform and less reproductible.
Trial registration number: Not applicable

P-453 Fertility preservation in endometriosis: Impact of the ovarian endometriosis and it's surgical treatment on oocyte yield

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Study question: Does a previous history of surgery for ovarian endometriosis (OMA) has an impact on controlled ovarian stimulation (COS) response in case of fertility preservation (FP) for endometriosis?
Summary answer: After COS, a prior history for OMA surgery was associated with poorer ovarian responsiveness compared to non-previously operated women.
What is known already: Endometriosis is a chronic disorder that affects 10% of woman, which can be responsible for infertility. The presence of OMA and/or it's excision could induce a reduction of the ovarian reserve (ROR), and for some women, an increased risk of premature ovarian failure. Therefore, FP with oocyte/embryo vitrification can be proposed for OMA-affected women, considering the relationship between endometriosis, infertility and ROR. Although a complete surgery excision of endometriosis lesions may be appropriate for some patients to relieve them from pain, the more efficient time to preserve fertility is still unknown in the management of women presenting OMA lesions.
Study design, size, duration: We conducted an observational multicentric study from April 2015 to December 2019, in two tertiary care university hospitals. Women presenting OMA or having a previous history of surgery for OMA that had performed a FP with COS for oocytes/embryo vitrification during the study period were included. Diagnosis of endometriosis was based on published imaging criteria using transvaginal sonography and magnetic resonance imaging or histologically proven in women who had past surgery.
Participants/materials, setting, methods: A total of 165 women were allocated to two groups, according to the presence of a previous history of surgery for endometrioma(s). Main outcome measure was the total number of oocytes retrieved.
Main results and the role of chance: Fifty-one (30,9%) women were included in the group 'previous history of surgery' and 115 (69,1%) in the group 'no history of surgery'. Mean age was 31,6±4,4 years and was not significantly different between groups (p=0.09). However, women in 'No previous surgery' group had higher AMH levels than women in 'previous surgery' group (2.27±1.70ng/ml versus 1.56±1.89ng/ml; p<0,001). In the group 'previous history of surgery', 21(41.2%) women had a recurrence of OMA(s) and 31 (60.8%) had at least one deep infiltrating endometriosis (DIE) lesion at FP. In the group 'no history of surgery', 92(80.7%) of the women had DIE. In addition, women in 'No previous surgery group' had larger OMA than women in 'previous surgery' group (mean diameter size: 5.56±4.34cm versus 3.25±2.16cm, respectively; p:0,03).

The mean number of COS with oocyte-retrieval was significantly higher in the group 'previous history of surgery' (2.0±1.02 versus 1.65±0.82 in the group 'no surgery', p=0.03), however, the total number of oocytes retrieved per women was significantly higher in women 'history of surgery', compared to women 'no previous surgery' (13.7±8.4 versus 10.3±7.5, p=0.02). In addition, the cancellation rate per cycle was significantly lower in 'No previous surgery' group compared to the 'previous surgery' group (0.09±0.31 versus 0.28±0.53; p<0.001).
Limitations, reasons for caution: No data concerning the thawing of oocytes/embryo are available for now.
Wider implications of the findings: FP is an essential component to integrate in ovarian endometriosis-management and should be proposed before surgery to optimize oocyte yield.
Trial registration number: not applicable

P-454 Oocyte vitrification for fertility preservation in women with benign gynecological disease: French national clinical guidelines with a modified Delphi consensus process

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Study question: Is there consensual clinical practices about fertility preservation (FP) for benign gynecological diseases BGD)?
Summary answer: A consensus study using the modified Delphi method identified 28 consensual practices concerning oocyte vitrification for fertility preservation in women with benign gynecological disease.
What is known already: Clinical Practical international guidelines are still published in oncology for offering standardized information and care for adults and children with cancer. Recently, the ESHRE Female Fertility Preservation Guideline Development Group published recommendations for healthcare professionals involved in fertility preservation for post-pubertal women and transgender adolescents and young adults. However, benign gynecological indications weren't distinctly individualized of malignant conditions. There's a lack of large cohort studies assessing the risks and outcome of FP for benign gynecological diseases. Healthcare professionals need consensus for defining the "good" indications of FP for benign gynecological diseases that could impair fertility.
Study design, size, duration: A steering group composed by 14 healthcare professionals and a patient representative with lived experience of endometriosis identified 42 potential practices concerning fertility preservation for benign gynecological disease. Then, 114 key stakeholders including various healthcare professionals (n=108) and patient representatives(n=6) were asked to answer at two rounds of a modified Delphi via an online survey from February to September 2020.
Participants/materials, setting, methods: Participants had to score 42 items for the first round and 31 for the second round using a nine-point Likert scale. These statements were distributed into five categories: Information to deliver to age-reproductive women with a BGD (n=9), technical aspect of fertility preservation for BGD (n=6), indications of FP for endometriosis (n=13), indications of FP for none-endometriosis BGD (n =10), idiopathic diminished ovarian reserve in the absence of gynecologic and endocrinologic diseases (n=4).
Main results and the role of chance: Survey response of stakeholders was 75 % (86 out of 114) for the round 1 and 87 % (75 out of 86) for the round 2. Consensus recommendations were achieved for 28 items, and no consensus between stakeholders was achieved in the remaining items. Stakeholders rated the importance of an age-specific information concerning the risk of diminished ovarian reserve after surgery and the necessity to inform about the benefice/risk balance of oocyte vitrification, in particular about the chance of live-birth according to the age at the time of oocyte vitrification. They endorsed oocyte vitrification as the reference FP technique for those benign indications. Experts rejected to determine lower and upper age limits in women for fertility preservation. FP shouldn't be offered in rAFS stages I and II endometriosis without endometriomas.
Limitations, reasons for caution: Experts were only French native speakers from France, and Belgium. It would have been interesting to conduct this survey with experts from other continents.
Wider implications of the findings: At our knowledge, we present here the first guideline s focusing on FP in women with BGD, following a designed scientific Delphi procedure. These guidelines could be useful for gynecologists to better inform women with benign gynecological diseases about the indication or not to offer a FP procedure.
Trial registration number: Not applicable

P-455 Breast cancer recurrence in women with and without controlled ovarian stimulation for fertility preservation. Cohort study.

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Study question: Do patients diagnosed with breast cancer who undergo ovarian stimulation for fertility preservation prior to chemotherapy have a higher risk of recurrence of the disease?
Summary answer: There was no statistically significant difference in the hazard ratio for breast cancer recurrence in fertility preservation-stimulated women compared to non-stimulated ones
What is known already: While many women with early breast cancer benefit from chemotherapy treatments in increasing disease-free survival, they are also at risk of permanent chemotherapy induced ovarian failure.

Oocyte cryopreservation with an adapted protocol with letrozole may reduce the possible deleterious effect of the hyper estrogenic state during the controlled ovarian hyperstimulation (COH). Although fertility preservation in women diagnosed with breast cancer seems safe, the follow-up periods of most studies are short in time. In addition, follow-up data of COH before neoadjuvant chemotherapy in women with hormone negative tumor receptors is still scarce and briefly reported.
Study design, size, duration: It was a retrospective cohort study, where 208 women with non-metastatic breast cancer were included. The recruitment period was from 01/01/2009 to 01/12/2019. The minimum follow-up period was 6 months, and the maximum, 130 months.

Participants were divided into two cohorts, those who received controlled ovarian hyperstimulation prior to their cancer treatment and those who did not. Patients were followed until disease recurrence, death, loss to follow-up, or end of the study
Participants/materials, setting, methods: Setting: university hospital in Buenos Aires, Argentina. We included women aged 18 to 45 years with a recent histological diagnosis of non-metastatic breast cancer who had to receive chemotherapy with gonadal toxicity. We excluded patients with a history of previous chemotherapy or radiotherapy for another cancer disease, or menopause. Follow-up was at least an annual clinical check-up and breast imaging.

Cohorts were analysed using a Cox-proportional hazards model, adjusted for propensity score for receiving stimulation.
Main results and the role of chance: We included 208 women, 39 in the COH group and 169 in the non-stimulated group (NSG). The only statistically significant difference was in age: median years 33.7 (interquartile range -IQR- 30.9 to 36.9) and 40.0 years (IQR 36.8 to 44.0), respectively. The median size of cancer nodules was 19.0 millimetres (IQR 10.0-30.0) and 17.0 (IQR 11.0-25.0), p 0.547; percentage of positive lymph nodes: 41.0% vs 39.3%, p 0.841; positive hormonal receptors: 84.6% vs 85.2%, p 0.925; percentage of neoadjuvant chemotherapy: 20.5% vs 11.4%, p 0.128. There were also no statistically significant differences regarding tumour stage, high Ki-67 labelling index, positive breast cancer genes (BRCA 1 or 2), and radiotherapy.

Overall, 18.0% of patients had cancer recurrence in the COH group and 20.7% in the NSG (p 0.699). Crude cancer recurrence rates were similar: 5.96 per 100 patients/year (95%CI 2.84-12.50), and 4.65 per 100 patients/year (95%CI 3.34-6.47), respectively. The crude hazard ratio (HR), comparing the COH group vs the NSG was 1.32 (95%CI 0.58-2.97; p 0.507). The adjusted HR using a propensity score for receiving ovarian stimulation treatment was 1.08 (95%CI 0.39-2.98; p 0.887). Results were similar if adjusted for age, neoadjuvant chemotherapy, and other confounders.
Limitations, reasons for caution: This was a single-center retrospective cohort study. There might be unknown or residual confounders that could influence results. Nevertheless, we accounted for treatment bias using a propensity score for ovarian stimulation. Results should be extrapolated with caution, especially in other non-university institutions and populations.
Wider implications of the findings: This study provides new evidence on the safety of controlled ovarian stimulation in breast cancer patients prior to

chemotherapy treatment, in a Latin American population. Letrozole continues to show safety and efficacy as an adapted protocol in breast cancer.
Trial registration number: not applicable

P-456 Impact of first-line chemotherapy prior to ovarian tissue cryopreservation on primordial follicle activation and survival in pre-pubertal and young adult patients

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Study question: How does chemotherapeutic regimen administered prior to ovarian tissue cryopreservation affect the signaling pathways regulating ovarian reserve and follicular survival in pre-pubertal and adult patients?
Summary answer: Chemotherapy induces key signaling processes of follicle activation and increases apoptosis in quiescent follicle. However, damages were different according to the age of the patient.
What is known already: Therapeutic regimen can damage the ovarian reserve leading to infertility of cancer survivors. Among fertility preservation options, cortical tissue banking appears to be an attractive alternative for patients who cannot delay their treatment or have already started chemotherapy. Though previous studies showed that first-line chemotherapy may induce follicular damages, the impact on follicle activation signaling pathways in human remains poorly understood. Deciphering the signaling modifications under chemotherapy is critical to have a better understanding of the follicle depletion process. Moreover, only few studies on cryopreserved tissue were conducted in children whereas follicle distribution differs compared to post-pubertal women.
Study design, size, duration: Cryopreserved ovarian tissue from young adult (16-27 years old, n=6) and pre-pubertal (3-10 years old, n=6) cancer patients were used as model. Fragments were thawed and cultured for 24 hours after size homogenization (4x2x0.5 mm). Patients who received chemotherapy before ovarian tissue cryopreservation were compared to non-exposed patients. PI3K/AKT/mTOR and Hippo pathways, as well as follicles and stroma survival, were assessed among the different groups at thawing and after culture.
Participants/materials, setting, methods: The impact of previous chemotherapy exposure on follicle activation, on the PI3K/AKT/mTOR and Hippo pathways was assessed at thawing and after 24 hours of culture by protein analyses (immunostaining and western blot). Histological analyses (follicular counting, immunostaining and TUNEL staining) were performed at the two timepoints to assess follicle distribution, morphology, stroma structure and apoptosis. Main results and the role of chance: The damage of chemotherapeutic regimen prior to cryopreservation was observed specifically on quiescent follicles after thawing by TUNEL staining in both adult and pre-pubertal patients. Surprisingly, apoptosis occurred more specifically in oocytes of pre-pubertal treated tissue while adult treated patients showed granulosa cells death. After culture, apoptosis was observed in the stroma but healthy follicles were observed in all conditions. Atretic follicles were observed similarly in pre-pubertal and adult cortex previously exposed to chemotherapy while not in the unexposed tissue. Protein analyses showed a higher expression of PI3K and Hippo proteins among all groups at thawing compared to cultured groups while difference was observed between pre-pubertal and adult cortex. At thawing, cortical tissues previously exposed to chemotherapy had a higher expression of phosphorylated forms of AKT and RPS6 compared to untreated groups, irrespective to the age. Moreover, immunostainings showed an oocyte-specific localization of p-AKT while p-RPS6 was more pronounced in the granulosa cells, suggesting an early process of follicle activation.
Limitations, reasons for caution: This study was limited to the evaluation of two major signaling pathways, PI3K/AKT/mTOR and Hippo. Moreover, considering the scarcity and the heterogeneity of our model, the number of patients included in this study is limited and the results should be interpreted with caution.
Wider implications of the findings: Our results highlight the involvement of age and previous chemotherapeutic treatment in the regulation of signaling pathways regulating follicular activation, growth, and survival. Besides sustaining the chemotherapy-induced "burn out effect" theory, it opens perspectives to regulate the deleterious impacts of chemotherapy on follicles though molecular control of the altered pathways.
Trial registration number: not applicable

P-457 Emergency fertility preservation (FP) in female patients-utilisation rate of the stored eggs/embryos and pregnancy outcomes.

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Study question: The aim of the study was to determine the percentage of patients returning to use their stored eggs/embryos following FP and their pregnancy outcomes.
Summary answer: The patient utilisation rate for eggs/embryos was 17% with a live birth rate of 59%.
What is known already: Fertility preservation is considered as a vital issue for individuals in the reproductive stage of life when their future fertility may be compromised. Increased cancer survival rate and advances in assisted reproductive techniques make this an essential service to offer to patients facing life limiting disease or long-term medical conditions.FP is important to improve the quality of life in cancer survivors.
Study design, size, duration: A retrospective analysis was performed over a period of ten years between January 2010 to December 2020 in our tertiary unit. A total of 75 patients who underwent FP were identified.
Participants/materials, setting, methods: Infertility database for embryology and andrology (IDEAS) was used for the data collection and analysis. Patient's age, reasons for fertility preservation, type of benign/cancer condition, protocol used for controlled ovarian stimulation (COS), dose of the gonadotropins, number of eggs collected, number of eggs/embryos cryopreserved, duration between storage and fertility treatment, pregnancy outcomes were included in the analysis.
Main results and the role of chance: Seventy-five patients underwent FP during the 10-year study period. The mean age was 30 years (range 17-43). Seventy-two patients (96%) underwent treatment for oncological reasons and the rest (4%) were for gender transition and Crohn's disease. The most common types of malignancies include breast cancer (36%), Hodgkin's lymphoma (18%) and cervical cancer (15%). Ninety-two percentage of patients underwent COS with an antagonist cycle, with an average of 10.8 eggs collected. Recombinant follicle stimulating hormone (FSH) was used in 92% of the cycles and human menopausal gonadotropin (HMG) was used in 8%. Fifty-eight percentage were given a maximum dose of 300IU of gonadotropin. The mean yield of eggs was higher in patients with breast cancer (12.62) followed by Hodgkin's (10.5) and cervical cancer (9.6). Majority (60%) had embryo cryopreservation (82% at blastocyst stage and 18% at day 3 cleavage stage) and the rest (40%) had egg cryopreservation. A total of 17% (12) of patients returned for treatment with a livebirth rate of 59% and miscarriage rate of 8%. One third of livebirths were achieved through surrogacy. The average duration between fertility preservation and return for treatment was 2.4 years.
Limitations, reasons for caution: During the last 5 years, there has been an increase in the number of young women requiring FP in our unit. These women may require a considerable amount of time to complete their oncological treatment before embarking on pregnancy using their stored eggs/embryos.
Wider implications of the findings: As cancer survival rate improves, there will be a likely increase in the utilisation rate for follow up treatment among young women who had FP.

The overall awareness of the gonadotoxic effect of cancer therapy and available fertility preservation options among both patients and clinicians needs to be increased.
Trial registration number: NA

P-458 A computational biology approach to improve in-vitro folliculogenesis

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Study question: Considering the complexity of mechanisms involved in mammalian ovarian folliculogenesis, how about improving the current *in-vitro* folliculogenesis (*iv*F) protocols to prolong individual reproductive chance?
Summary answer: Computational modelling approach based on network theory was used to manage complexity, improve *iv*F knowledge and discover new molecules to be targeted for innovating assisted-reproductive-technologies. What is known already: Over the past decades, based on the large ovarian-pool of immature-gametes availability, *iv*F systems were developed in several mammalian species to support oocyte growth in order to preserve human-fertility and contrast endangered species extinction. Only mouse live-births were obtained when primordial/primary follicles were cultured *in-vitro*, instead the oocyte differentiation is extremely slow in medium-sized mammals. Moreover, the degree of meiotic-competence is quite incomplete if compared to mice, because oocytes must proceed until late antral-follicle stage to acquire a complete developmental competence. These observations denote the importance to adopt further investigations for establishing a complete *iv*F protocol in translational mammal model.
Study design, size, duration: Two researchers expert on reproductive biology generated the Web of Science-Mammals-Made *in-vitro* folliculogenesis (WoS_MMivF) database including 1111 manuscripts published in peer-reviewed international papers indexed selected in Advanced Search of WoS "Core-collection" by carrying out an independent analysis. Two additional researchers verified the correctness of the records.
Participants/materials, setting, methods: WoS_MMivF network was built up using Cytoscape 2.6.3 software. The network was analyzed for topological parameters (closeness-centrality, betweenness-centrality and edge count) and to identify key controllers (Hub.BN). Bidimensional-kernel-density-estimation (2D KDE) identifies Hub.BN controllers; Search-Tool-for-the-Retrieval-of-Interacting-Genes/Proteins (STRING) were used to enrich the network with new proteins.
Main results and the role of chance: The analysis of topological parameters demonstrated that the network is scale-free according to Barabási-Albert-model with a high-degree of robustness-against-random-damage, great controllability and navigability. The network reproduces a coherent framework identifying cross-talking molecules playing a key role in the inter-follicular/intra (somatic and germinal compartment) dialogue.

The network allows to organize signalling transduction events/molecules by stratifying them in three layers: input-layer recognizes molecules generating the information flux working as systemic endocrine (pituitary/chorion/enteric-related endocrine hormones) and local paracrine-factors (TGFbeta-superfamily-members and growth-factors) exerting either intrafollicular control or remote feedback on reproductive-cycle. Processing-layer presents molecules able to elaborate/amplify the endocrine/paracrine controllers of ovarian functions, including components of codified intracellular-signaling-pathways like PI3K, KIT and MAPK and second messengers cAMP and Ca2+. These cascades are necessary to promote *in-vitro* reproducible follicular functions and modulate steroidogenesis, representing molecular events stratified in the output-layer.

STRING analysis allowed to extend the regulatory flow of information towards two major biological action contexts: metabolic-control (paracrine-factors and signal-transduction) and angiogenesis. Metabolic-control mediated by mTOR and its interactor cognates FOXO1, FOXO3/SIRT1 plays a key role for *iv*F, representing the energy sensors of the reproductive cells in hypothalamic-pituitary-ovarian-axis first regulating the status of follicle quiescence/activation and then fate of the structure (specialization or apoptosis).
Limitations, reasons for caution: -
Wider implications of the findings: STRING identified mTOR as key pathway of folliculogenesis, which might act as a molecular-switch to be pharmacologically targeted for potential new *in-vitro* strategies modulating follicular fate. These results suggest that computational approach in biology might offer perspective in identifying unknown signals, implementing research questions and innovative protocols to face female-fertility.
Trial registration number: not applicable

P-459 Ex vivo perfusion of whole ewe ovaries with follicular maturation for up to seven days: towards the development of an alternative fertility preservation method

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Study question: To develop an alternative fertility preservation method for young female cancer patients based on an *ex vivo* perfusion of whole ovaries serving as a platform for future ovarian stimulation studies.
Summary answer: It is possible to maintain viable follicles and to retrieve oocytes after *ex vivo* perfusion of ewe ovaries for up to 7 days.
What is known already: Some progress has been made in terms of follicular growth and the isolation of mature oocytes *in vitro*. However, full development, from early follicular stages to a viable offspring, has only been described in rodent models. The complex events controlling follicular expansion and the long time required for folliculogenesis and oocyte maturity in large mammalian species creating challenges and limitations for *in vitro* studies. E*x vivo* perfusion of a whole ovary could potentially be a solution by exploiting the intact ovarian architecture to support folliculogenesis and oocyte maturation.
Study design, size, duration: Thirty-one ewe ovaries were divided into 4 groups and *ex vivo* perfused in a bioreactor. Group 1 (n=14) perfusion for 48 hours with no hormone supplementation; Group 2 (n=4) perfusion 96-101 hours with follicle stimulating hormone (FSH); Group 3 (n=3) perfusion 120-168 hours with human menopausal gonadotropin (hMG); Group 4 (n=10) perfusion 72-144 hours with hMG.
Participants/materials, setting, methods: Ewe ovaries from sexually mature ewes were *ex vivo* perfused in a bioreactor under normothermic conditions for up to 7 days (max total 168 hours). Histomorphological, immunohistochemical, hormonal and biochemical analyses were performed to assess ovarian structure and viability after cold ischemia and after perfusion which was subsequently compared to control ovaries.
Main results and the role of chance: The perfused ovaries in group 2 and 3 showed no significant differences in follicular density, viability and oocyte quality after ischemia and perfusion compared to control ovaries. Estradiol and progesterone levels did not increase during the perfusion. The perfused ovaries in group 1 and 4 showed a significant decrease in the ovarian reserve and oocyte quality. In total, 16 GV-MI oocytes were retrieved from groups 3 and 4.
Limitations, reasons for caution: 1. Ovaries were retrieved from ewes of unknown cycle and reproductive history. 2. The perfusion medium was changed after 24 hours from perfusion start to remove detrimental metabolites and this could affect the measured concentrations of hormones and metabolites in the perfusion medium.
Wider implications of the findings: These results pave the way to propose *ex vivo* perfusion as a good platform for fertility preservation studies on whole mammalian and human ovaries to retrieve fully mature oocytes.
Trial registration number: Not applicable

P-460 Impact of various cancers on semen parameters in a tertiary onco-fertility unit in India

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Study question: This study evaluated differences in semen parameters in male cancer patients in our ethnic population who banked their sperms prior to cancer treatment

Summary answer: We found significant differences in semen concentration, motility and morphology between different types of cancers, especially testicular cancers

What is known already: Impaired spermatogenesis and abnormal semen parameters in cancer patients has been noted, however certain cancer types are more damaging than others. In testicular cancer, spermatogenesis impairment is more quantitative than qualitative with sperm morphology being the most affected parameter. Among non testicular cancers, lymphoma cases usually show the most significantly impaired semen parameters

Study design, size, duration: We conducted a retrospective study analyzing semen parameters in 49 cancer patients between October 2014 to January 2020 who presented to the onco-fertility unit, Max Multispeciality Hospitals, New Delhi.

Furthermore, we did our analysis based on total of 101 samples and were broadly divided into testicular (37 samples) and non testicular cancers (64 samples). Patients who had previously received any form of cancer treatment including chemotherapy or radiotherapy were not included in this study

Participants/materials, setting, methods: Testicular Cancer(TC) group was further subcategorized into Seminoma and Non Seminoma groups whereas Non Testicular Cancer (NTC) group was subcategorized into Lymphoma and Non Lymphoma groups. Semen was collected by masturbation and analysis was performed in keeping with the WHO criteria. Statistical analyses was performed using SPSS software. p values <0.05 were considered to indicate statistical significance.

Main results and the role of chance: In Testicular cancer (TC), 92% samples (34/37) had abnormal semen parameters whereas only 24.4% samples (22/64) were abnormal in Non Testicular cancer (NTC). Additionally, there were significant differences in sperm concentration, motility and morphology between TC and NTC groups.

Individually,

TC: Oligozoospermia was seen in 73% (27/37) with subdivision between Seminoma and Non Seminoma groups being 81.3% (13/16) and 61.9% (13/21).

Asthenozoospermia was seen in 86.5% (32/37) samples with subdivision between Seminoma and Non Seminoma groups being 87.5% (14/16) and 81% (17/21).

Teratozoospermia was seen in 59.5% (22/37) samples with subdivision between Seminoma and Non Seminoma groups being 75% (12/16) and 42.86% (9/21).

Combined OATS observed in 59.5% (22/37) samples with subdivision between Seminoma and Non Seminoma groups being 75% (12/16) and 42.86% (9/21)

NTC: Oligozoospermia was seen in 18.8% (12/64) samples with subdivision between Lymphoma and Non Lymphoma groups being 26.92% (7/26) and 26.32% (10/38).

Asthenozoospermia was seen in 32.8% (21/64) samples with subdivision between Lymphoma and Non Lymphoma groups being 34.62% (9/26) and 34.21% (13/38).

Teratozoospermia was seen in 17.2% (11/64) samples with subdivision between Lymphoma and Non Lymphoma groups being 26.9% (7/26) and 23.68% (9/38).

Combined OATS observed in 17.2% (11/64) samples with subdivision between Lymphoma and Non Lymphoma groups being 26.9% (7/26) and 23.68% (9/38).

Limitations, reasons for caution: Study was conducted in a single institution with lesser overall number of patients. Duration, staging and grading of cancers were also not individually assessed, which could be a further limiting factor.

Wider implications of the findings: Testicular cancers, especially seminomas, have the most severe effect upon semen parameters. Among NTC patients, lymphomas have the worst impact. Knowing the varying effect of different cancers on semen parameters in our ethnic population helps ART specialists and oncologists to appropriately modify patient counseling and improve fertility outcomes.

Trial registration number: RMO13019

P-461 A 16-year bicentric retrospective analysis of ovarian tissue cryopreservation (OTC) in paediatric patients: indications, results and outcome

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Study question: What is the outcome of ovarian tissue cryopreservation (OTC) in paediatric patients from the beginning of its setting in two different French centres?

Summary answer: In our cohort of 75 paediatric patients who underwent OTC, the mean age, malignancy rate and survival rate were 9.7 years, 70.7% and 77.3% respectively.

What is known already: Cancer treatments of last decades improve the survival rate of children and adolescents; however chemo- and radiotherapy result in gonadal damage leading to acute ovarian failure and sterility. The preservation of fertility is now an integral part of care of children requiring gonadotoxic treatments.

Currently OTC represents the only possibility of preserving the potential fertility in prepubertal girls. OTC is an effective fertility preservation option which allows long-term storage of primordial follicles, subsequent transplantation restores endocrine function and fertility. The efficacy of these techniques is well-demonstrated within adult population but the data are poor for paediatric patients.

Study design, size, duration: This is a retrospective study of OTC practice of two French centres from January 2004 to May 2020.

Participants/materials, setting, methods: A total of 75 patients from paediatrics units underwent cryopreservation of ovarian tissue before gonadotoxic therapy for malignant or benign diseases. The ovarian cortex was cut into fragments and the number of follicles per square millimeter was evaluated histologically. The long-term follow-up includes survival rate, hormonal and fertility status.

Main results and the role of chance: The mean age at OTC of 75 patients was 9.7 years [0.2 – 20], 32% were postpubertal. 53 had malignant disease and 22 had non-malignant disease. The most frequent diagnoses in this cohort included acute leukemia, hemoglobinopathies and neuroblastoma. Indication for OTC was stem cell transplantation for 78.7% (n=59) girls.

A third of each ovary was collected for 62,7% (n=47) patients, a whole ovary for 33,3% (n=25) patients and a third of one ovary alone for 4,0% (n=3) patients. An average of 17 fragments [5-35] per patient was cryoconserved. A correlation was found between age and the number of fragments ($p < 0.001$). More fragments were obtained from partial bilateral harvesting than from whole ovary harvesting ($p<0.05$). Histological analysis of ovarian tissue showed a median of 6.0 primordial follicles/mm2 [0.0–106.5] and no malignant cells were identified. A negative correlation was found between age and follicular density ($p<0.001$).

Median post-harvest follow-up was 92 months [1–188]: 17 girls had died, 12 were still treated for their pathology and 46 were in complete remission. Of all patients, 29 have been subject to hormonal status evaluation and 26 were diagnosed with premature ovarian insufficiency ($p<0.001$). One patient had undergone thawed ovarian tissue transplantation.

Limitations, reasons for caution: This study is a retrospective analysis. The cohort was not compared with a control group who did not undergo OTC or with an adult population. Furthermore, many of these girls are still young and do not intend to use the transplantation of thawed ovarian tissue yet.

Wider implications of the findings: OTC should be proposed to all girls with high risk of developing premature ovarian insufficiency following

gonadotoxic therapies in order to give them the possibility of fertility and endocrine restoration.
Trial registration number: Not applicable

P-462 First live birth after fertility preservation using vitrified oocytes in a woman with mosaic Turner syndrome

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Study question: Is oocyte vitrification an option for preserving the fertility of women diagnosed with Turner syndrome (TS)?
Summary answer: We report the first live birth achieved using cryopreserved oocytes in a woman diagnosed with mosaic Turner syndrome.
What is known already: Women with TS are at extremely high risk for premature ovarian insufficiency (POI) and infertility. Although the desire of becoming parents may be fulfilled through egg donation or adoption, fertility preservation using ovarian tissue cryopreservation or oocyte vitrification has been offered to adolescents with TS before complete exhaustion of their follicular stockpile. However, women with TS exhibit higher rates of pregnancy loss and obstetric complications, and the feasibility of fertility preservation in TS is hampered by the reduced follicular pool and by concerns about the X chromosomal content of oocytes and follicular cells.
Study design, size, duration: Case report in a university hospital.
Participants/materials, setting, methods: A 25-year-old woman with Turner syndrome mosaicism (45,X0[14]/46,XX[86]) was referred for fertility preservation (FP) counseling. Serum antimüllerian hormone (AMH) level was normal (6.4 µg/L). In view of parenthood postponement and because of the unpredictable rate of follicle loss, the woman underwent two cycles of ovarian stimulation using recombinant follicle stimulating hormone (rFSH), 200-250 IU/day for 8 resp. 12 days, in a GnRH antagonist protocol.
Main results and the role of chance: In total, 29 metaphase II oocytes (MII) were vitrified. Five years later, the patient returned to the clinic with a desire for pregnancy. Because of evidence of considerable AMH decline (-56% in an interval of four years), the patient was advised to utilize her cryopreserved oocytes for in-vitro fertilization with preimplantation genetic testing for aneuploidy screening (PGT-A). All 29 MII oocytes were thawed; 26 oocytes survived (89.7%) and were inseminated using intracytoplasmic sperm injection (ICSI). Thirteen oocytes were fertilized normally. Three good quality blastocysts ensued and were vitrified after trophectoderm biopsy for PGT-A using array-CGH. Two blastocysts were found euploid. One was thawed and transferred into the uterus using a HRT priming protocol. An uneventful pregnancy occurred. The patient delivered a healthy baby girl weighing 3490 g at 40 weeks of gestation.
Limitations, reasons for caution: Cryopreservation of oocytes and/or ovarian tissue in selected postmenarchal girls or young women with Turner syndrome is an investigational FP approach that may result in genetic parenthood. The feasibility of FP in TS individuals is limited to those with evidence of ovarian function, before POI occurs.
Wider implications of the findings: Cryopreservation of mature oocytes after ovarian stimulation is a realistic option for FP in selected postmenarchal individuals with mosaic TS. Whether PGT-A may reduce the risk of pregnancy loss in TS has to be confirmed by further studies.
Trial registration number: not applicable

P-463 Patients undergoing elective and onco-fertility preservation respond similarly to controlled ovarian stimulation for fertility preservation

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Study question: Is the oocyte vitrification response different in patients undergoing elective and onco-fertility preservation?
Summary answer: Patients undergoing elective and onco-fertility preservation seem to respond similarly to controlled ovarian stimulation for fertility preservation.
What is known already: Age persists as the factor with the most significant impact on the prognosis of female fertility. The ovarian reserve can also be threatened by surgical, radiotherapy or chemotherapy procedures. Thus, maternity delay and the increased incidence of malignant diseases are the most jeopardizing conditions for reproductive potential in women. Studies are still conflicting about oocyte freezing results in patients with and without cancer. Some studies suggest worse outcomes in patients with cancer regarding the number of mature vitrified oocytes when compared to healthy patients whether others show similar response to the ovarian stimulation for fertility preservation in both groups.
Study design, size, duration: Observational, cross-sectional, and historical study using data from 367 who underwent oocyte vitrification from a Reproductive Medicine Center, between 2009 and 2018.
Participants/materials, setting, methods: Patients were divided into an elective group (EG; n = 327) and an onco-fertility group (OFG; n = 40). Data were presented as mean ± standard deviation or median and interquartile range (IQR) and absolute and relative frequencies. Chi-square test, Student's t-test, or Mann-Whitney test were applied. Generalized linear models were used to control confounding factors. Data were adjusted by women age, FSH, and GnRH protocol. The null hypothesis was rejected when p <0.05. Main results and the role of chance: Patients age in OFG was significantly lower compared to EG (31.3±5.8 vs. 37.0 ±2.9 years; p <0.01) and also FSH measurement (4.0 [3.3 – 6.2] vs. 9.0 (5.4 – 9.9) mIU/mL; p <0.01). The presence of a partner was significantly higher in OFG (25 [62.5%] vs. [19.9%]; p<0.001). GnRH antagonist protocol was used in 80.1% of cycles, and FSH-r was used in 80.4% of cycles. Letrozole was added for 20 breast cancer patients (74%). When adjusting data for age, FSH and Gonadotropin-releasing Hormone (GnRH) protocols, no significant difference in the number of vitrified mature oocytes between the two groups were observed (6.0 [3.0–11.0] vs. 7.0 [3.0–12.0]; p=0.11). Limitations, reasons for caution: The number of women in the OFG was lower than the EG group. The OFG was composed of different types of tumors in different locations and stages. Thus, it can be questioned whether any patient with a more aggressive tumor might have had a negative impact on the results.
Wider implications of the findings: Healthy patients and patients with cancer seem to respond similarly to ovarian stimulation for fertility preservation. The extensive number of cycles performed for EG in contrast to OFG leads to a reflection on patients who are still not referred for reproductive counseling after a cancer diagnosis.
Trial registration number: not applicable

P-464 What is the optimal ovarian stimulation (OS) protocol for women who undergo planned oocyte cryopreservation (POC)?

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Study question: When repeated cycles of OS for planned oocyte cryopreservation using a standard GnRH antagonist protocol are required, can OS protocol modifications improve oocyte yield?
Summary answer: Compared to repeating a standard GnRH antagonist protocol, switching to a long GnRH agonist protocol for POC results in a higher number of cryopreserved oocytes.
What is known already: The total number of cryopreserved oocytes is a key parameter of POC programs because of its association with livebirth. A substantial proportion of women embarking on POC will undergo repeated cycles of OS to reach their desired target number of vitrified oocytes. According to recent guidelines, the GnRH antagonist protocol with GnRH agonist triggering is considered the first choice protocol for POC, because of its safety profile and convenience. However, in women with normal ovarian reserve, the long GnRH agonist protocol results in a higher number of oocytes retrieved. Evidence regarding the optimal protocol for POC is limited.
Study design, size, duration: This is a single-centre, retrospective cohort study including 283 women who had a first cycle for POC using a standard GnRH antagonist protocol and who requested a second OS cycle to increase their total

number of vitrified oocytes for later use. The choice of protocol for the second cycle was left at the discretion of the reproductive medicine specialist. All OS cycles took place between January 2009 and December 2019 in a tertiary referral hospital.

Participants/materials, setting, methods: After ovarian reserve testing, the first cycle OS was performed using rFSH or HPhMG in a GnRH antagonist protocol. For the second cycle, a GnRH antagonist protocol with or without antagonist pretreatment, or a long GnRH agonist protocol was prescribed. The primary outcome was the number of mature oocytes (MII) vitrified per cycle. Cycle characteristics were compared. Data were assessed by generalized estimating equation (GEE) regression analysis adjusting for covariates.

Main results and the role of chance: In total, 226 (79.9%) women had a GnRH antagonist protocol and 57 (20.1%) had a long GnRH agonist protocol in their second OS cycle for POC. Overall, mean age was 36.6±2.4 years. The median (CI) number of mature oocytes vitrified after the second OS cycle was significantly higher than that after the first cycle [8 (5-11) vs. 7 (4-10), p<0.001]. According to GEE multivariate regression, adjusting for relevant confounders, switching from a GnRH antagonist protocol in the first cycle to a long GnRH agonist protocol in the second cycle was the only significant predictor of the number of vitrified oocytes after the subsequent cycle (coefficient 1.59, CI 0.29-2.89, p-value = 0.017). Age, AFC, initial dose and type of gonadotropins did not predict the number of vitrified oocytes. None of the women developed moderate or severe OHSS.

Similarly, of 174 women who underwent their first OS cycle with a standard GnRH antagonist protocol, 133 women (76.4%) had the same protocol for their second cycle and 41 women (23.6%) an additional three-day course of GnRH antagonist pretreatment. According to GEE multivariate regression, this protocol modification did not result in more mature oocytes available for vitrification (coefficient -0.25, CI -1.86-1.36, p-value = 0.76).

Limitations, reasons for caution: These data should be interpreted with caution because of the retrospective design and limited sample. Although more oocytes were obtained with a long GnRH agonist protocol we have no data on livebirth in women returning to use their oocytes to support the choice for a specific OS protocol for POC.

Wider implications of the findings: Although oocyte yield in the context of POC is an important parameter that may be modulated by the choice of OS protocol, the ultimate outcome measure of a successful POC program is livebirth after oocyte vitrification. Future research of oocyte parameters reflecting oocyte quality is paramount.

Trial registration number: not applicable

P-465 Effect of letrozole over Ki-67 expression in breast cancer during controlled ovarian stimulation (COS) for fertility preservation (FP): case report

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Study question: Does concomitant letrozole administration during COS alter Ki-67 expression in women undergoing FP procedures before breast cancer surgery?

Summary answer: Concomitant letrozole administration during COS, even for a short period, can reduce Ki-67 expression in breast cancer.

What is known already: The biggest concern with COS in breast cancer patients is the increase in serum estradiol levels, caused by the development of multiple follicles simultaneously. This has always been a major hindrance to the use of traditional ovarian stimulation regimens in these patients, due to the large amount of evidence on the pathogenetic role of estrogen in breast cancer propagation. To limit the rise of estradiol during COS, most centers have adopted concomitant letrozole administration. Recently, some studies have reported changes in tumor pathology after letrozole administration, such as a significant fall in Ki-67 expression.

Study design, size, duration: Case report including 2 patients undergoing COS with concomitant letrozole administration for 12 days before breast cancer surgery.

Participants/materials, setting, methods: The first patient was a 28-year-old Caucasian woman with a breast biopsy showing an infiltrating ductal carcinoma in the upper external quadrant of the right breast. The second patient was a 33-year-old Caucasian woman with a diagnosis of infiltrating ductal carcinoma of the upper external quadrant of the left breast. Both patients underwent COS with concomitant letrozole administration 5 mg daily for 12 days. Ovarian stimulation was performed using a GnRH-antagonist random-start protocol.

Main results and the role of chance: In the first patient, Ki-67 expression in the initial biopsy was 55%. After completion of FP procedures, she underwent quadrantectomy with sentinel-lymphnode biopsy. In the final histopathological report Ki-67 expression fell to 25%. In the second patient, the first biopsy showed a Ki-67 expression of 30%, while after mastectomy it fell to 10%.

Limitations, reasons for caution: Only 2 patients were included in the study.

Wider implications of the findings: COS is feasible before breast cancer surgery, as long as an adequate cancer biopsy with immunohistochemical evaluation has been collected. Cytological diagnosis is not enough to start FP procedures. Evaluation of biological parameters after letrozole administration could lead to underestimation of cancer proliferation rate and to inappropriate treatment strategies.

Trial registration number: na

P-466 Fertility preservation for medical reasons: International and intra-national variation in provision and the gap between guideline and practice

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Study question: What is the gap between guidance and practice of fertility preservation between countries and within countries with common clinical guidelines?

Summary answer: Substantial variation in provision of FP exists between countries and within individual countries with gaps between national and international guidelines and policies governing provision.

What is known already: A robust guideline on female FP was published by ESHRE in 2020, advising the application of FP in cancer and other conditions where treatment with cytotoxic agents or surgery will compromise reproductive function. Across Europe, in 13 countries (43.3%) FP is funded for all available FP procedures, in 13 countries (43.3%) no FP funding is available, and in 4 countries (13.3%) at least one FP option is funded. Variation in state provision of fertility care in different countries in Europe was highlighted in the ESHRE guidance. It did not specifically examine individual national policies or whether a national policy exists.

Study design, size, duration: Five clinicians performing FP in Europe were contacted to collect current FP provision data. Policies retrieved from the internet were not included as they could not be verified. Finally, FP funding policies for 135 Clinical Commissioning Groups (CCGs) in England, 14 Health Boards in Scotland, 7 Health Boards in Wales and 5 Trusts in Northern Ireland and 17 policies for regional heath services in Spain were included were included.

Participants/materials, setting, methods: Policies on FP for the UK and Spain were reviewed (n=178), including policies from the 161 regions from the four nations of the UK and policies of 17 autonomous bodies in Spain. Information on funded procedures, type of conditions included for funding and duration of storage were extracted. The provision of FP was compared to the current European Society of Human Reproduction and Embryology (ESHRE) and National Institute for Health and Care Excellence (NICE) guidelines.

Main results and the role of chance: In England, 127/128 (99%) CCGs fund cryopreservation of oocytes, sperm and embryos. Cancer is the exclusive indication in 11%. Provision of FP for transgender individuals is specified in 28%, ovarian tissue cryopreservation is funded in 8% and storage funding varies from five to ten years.

In Scotland, a national policy is applied. All 14 health boards equitably fund cryopreservation of oocytes, sperm, embryos and ovarian and testicular tissue. Funding is provided for cancer, medical conditions which may impair fertility and transgender individuals. Storage funding is based on a five yearly review until age 43 in women and 60 in men. In Wales and Northern Ireland, cryopreservation of oocytes, sperm and embryos is funded for people undergoing medical or

surgical treatment that is likely to make them infertile, provision for transgender individuals is not specified and ovarian tissue cryopreservation is not funded.

In Spain, all 17 Health Services fund cryopreservation of oocytes, sperm and embryos for patients whose fertility is at risk due to gonadotoxic treatments or other pathological processes. Ovarian tissue cryopreservation is funded in 94%, provision for transgender individuals is specified in 12%, and storage funding is available until the age of 50 in women and 55 in men.

Limitations, reasons for caution: Inability to retrieve fertility preservation policies for every country in Europe is a limitation, for which ongoing collaboration is sought. The variable nature of FP provision is likely to be multi-factorial; a lag in publication of guidelines and updated policies, ethical considerations and resource distribution may govern health policies.

Wider implications of the findings: The study highlights that provision of FP not only varies between countries but is also inconsistent within the same country. It is clear that there is a gap between ideal, evidence-based practice and actual provision. Variation in policies limits uniform access to care for patients.

Trial registration number: Not applicable.

POSTER VIEWING
NURSING AND MIDWIFERY

P-467 Emotions, Thoughts, and Coping Strategies of Women with Infertility Problems on Changes in Treatment during Covid-19 Pandemic: A Qualitative Study

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Study question: What are the emotions, thoughts and coping strategies of women with infertility problems on changes in treatment during the COVID-19 pandemic?

Summary answer: Treatment-related procedures keep changing directions, exposing the women to high level of uncertainty. Changes in treatments may be perceived as threats to achieving parenting goals.

What is known already: Both infertility and the treatment process constitute a stressful experience. Literature reports that couples describe infertility as the most difficult challenge to overcome in their lives. In addition, it has been reported that women experience more anxiety, stress, and depression than men during this period. Societies and individuals affected by large-scale disasters, such as global pandemics, can develop stress-related disorders. Current data indicate that closure of fertility clinic during the COVID-19 pandemic was associated with a sharp increase in the prevalence of anxiety and depression among patients undergoing fertility treatments and was perceived as an uncontrollable and stressful event.

Study design, size, duration: The research was designed as a qualitative study. The data were collected from two Internet forums between October - December 2020. Blogs most frequently used by women with infertility in Turkey were simultaneously selected. The comments of 30 women were included.

Participants/materials, setting, methods: Data were screened by using the directed qualitative content analysis. After selecting the blog, emotions, thoughts, and coping strategies expressed by 30 women whose treatment was canceled due to the Covid-19 pandemic or who continued treatment during this period were included in the analysis. The themes created were adapted to Lazarus and Folkman's Transactional Model of Stress and Coping.

Main results and the role of chance: The thematic analysis of the expression of women with infertility problems in accordance with the Transactional Model of Stress and Coping stages of Lazarus and Folkman resulted in 4 themes: psychological changes, cognitive changes, changes in social life, and coping strategies. Some women perceived changes in treatments positively, and stopping the treatments due to the uncertainty of the pandemic and its effect on pregnancy and the baby made them feel safe. The majority of women appraised the closure of fertility clinics negatively impacted their lives. They experienced despair, uncertainty, disappointment, anxiety, anger, sadness, and exhaustion from waiting. Also, some participants did not find it right to delay the treatments and felt that the healthcare personnel postponed the treatments to avoid infection. Women experienced feelings of anger, distrust, and threats toward the health authorities. Moreover, the women in our study stated that they were always at home due to the pandemic, far from friends and family, and therefore did did not feel need for self-care and considered themselves ugly. The expressions of women mostly include emotion-based coping strategies. They used activities such as praying, exercising, distracting, noticing the positive side of postponing, and stopping treatments during the pandemic, accepting, and meditating.

Limitations, reasons for caution: Clinics closed due to the pandemic or limited procedures caused fewer women to come to the clinics. At the same time, it is not accepted for anyone other than working in the clinic to come to the clinics for scientific studies. Therefore, comments of women have been reached through blogs.

Wider implications of the findings: It is believed that approaches based on Lazarus and Folkman's model helped the health professionals to determine potential stressors for women with infertility during the pandemic, and identified areas that required strengthening and improved personal coping strategies.

Trial registration number: not applicable

P-468 Fertility-related quality of life in subfertile women undergoing transvaginal hydrolaparoscopy versus hysterosalpingography as a first-line tubal patency test

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Study question: Is there a difference in fertility-related quality of life (QoL) in subfertile women undergoing transvaginal hydrolaparoscopy (THL) versus hysterosalpingography (HSG) as a first-line tubal-patency test?

Summary answer: In subfertile women undergoing first-line tubal patency testing, THL and HSG resulted in comparable fertility-related QoL.

What is known already: Both subfertility itself and subfertility treatment can have a significant impact on QoL. Tubal patency testing as part of fertility work-up is also known as a potential stressor. Pain scores for THL are significantly lower than for HSG (VAS 4.7 vs 5.4 ;
[5]D:2.5, p 0.038), but acceptability of the procedures was found to be comparable. Fertility-related QoL has not yet been studied in women undergoing tubal patency testing.

Study design, size, duration: We used data from a randomised clinical trial performed in 4 Dutch teaching hospitals, NTR3462. Between May 2013 and October 2016, we randomly assigned 300 subfertile women to THL or HSG with live birth as primary outcome. We performed a standardized questionnaire study as part of a randomised controlled trial comparing THL and HSG in the work-up for subfertility.

Participants/materials, setting, methods: Women were eligible if they were undergoing a fertility work-up with an indication for evaluation of tubal patency testing. Fertility-related QoL was measured six weeks after the procedure with the validated FertiQoL questionnaire, which produces a Core (total) score and four subscale domains: Emotional, Relational, Social, and Mind-Body. FertiQoL scores for the Core score and subscales between THL and HSG were compared using Mann-Whitney-U test and multiple linear regression analysis.

Main results and the role of chance: We allocated 149 women to THL and 151 to HSG. The questionnaire was completed by 84 women in the THL group (response rate 56%) and 96 women in the HSG group (response rate 64%). Core scores were 74.6 ±12.8 for THL and 73.4 ±12.4 for HSG (p=0.39). Scores for the Emotional domain were 64.5 ±19.0 for THL versus 66.0 ±16.3 (p=0.67) for HSG. Scores for the 'Mind-body' domain for THL were 76.9 ±15.6 versus 74.1 ±18.0 for HSG (p=0.42), scores for the Relational domain were 79.2 ±12.9 for THL and 76.9 ±15.6 for HSG (p=0.21). Scores for the Social

domain for THL were 77.9 ±15.1 versus 76.7±14.1, (p=0.42). The optional 'Treatment FertiQol' was completed by 156 women. Total scores were 77.5 ±12.1 for THL versus 73.8 ±13.1 (p=0.08) for HSG. The multiple linear regression analysis showed only a statistical significant positive effect of higher age on the score for the Emotional domain (B:0.90, p=0.015).
Limitations, reasons for caution: One of the main limitations of this study was a response rate of 60%. Although this is considered an acceptable rate within questionnaire research, this could lead to selection bias.
Wider implications of the findings: In subfertile women, tubal patency testing with THL versus HSG did not result in differences in fertility-related QoL.
Trial registration number: NTR3462

P-469 Period Tracker Applications – are they giving women accurate menstrual cycle information?

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Study question: Are period trackers giving women accurate information about their periods and ovulation?
Summary answer: The top 10 period trackers gave conflicting information on period dates, ovulation day and the fertile window.
What is known already: Period tracking applications allow women to track their menstrual cycles and receive a prediction for their periods. The majority of applications also provide predictions of day of ovulation and the fertile window. Previous research indicates applications are basing predictions on assuming women undergo a textbook 28-day cycle with ovulation occurring on day 14 and a fertile window between days 10 and 17.
Study design, size, duration: An audit of menstrual cycle apps was conducted on the Apple app store using menstrual cycle tracker/period tracker as the search terms. The top ten apps that followed the inclusion and exclusion criteria were analysed and used for this study. All apps had the ability to allow retrospective data entry giving future cycle predictions and fertile window, and nine of the apps predicted a day of ovulation.
Participants/materials, setting, methods: Five women's profiles for 6 menstrual cycles were created and entered into each app. Cycle length (CL) and ovulation day (OD) for the 6th cycle were; Woman 1 – Constant 28 day CL, 0D 16, Woman 2 – Average 23 day CL, OD 13, Woman 3 – Average 28 day CL, OD 17, Woman 4 – Average 33 day CL, OD 20 and Woman 5 – Irregular, average 31 day CL, OD 14.
Main results and the role of chance: For cycle length, the apps all predicted woman 1's cycles correctly but for women 2-5, the apps predicted 0 to 8 days shorter or longer than expected. For day of ovulation; for woman 1, no apps predicted this correctly; the apps ranged from day 13-15. For woman 2, 1 app was correct and overall the apps showed a lot of variation from day 8 to 13. For woman 3, no apps got it right, with a range of day 13-16. For woman 4, 2 apps got it right, but the apps ranged from day 13-20. For woman 5, no apps got right; the apps ranged from day 13-21. Irrespective of cycle length, 7 apps predicted a fertile window of 7 days in almost all cases; except 1 app that predicted 6 days for woman 2 and a different app which predicted 8 days for woman 4. For the remaining 3 apps, one always predicted a 10 day fertile window. One app predicted an 11 day fertile window in 4/5 women. One app predicted a 12 day fertile window in 4/5 women.
Limitations, reasons for caution: The five profiles created spanned a range of observed cycle characteristics, but many permutations are possible. A Monte Carlo type analysis could be conducted to examine these possibilities to provide more precise assessment of app performance, but as data had to be added manually into apps, this was not possible.
Wider implications of the findings: The apps do not use the same algorithm and show variation. The information given by these apps is not 100% accurate, especially for the day of ovulation and the fertile window which can only be predicted if using a marker of ovulation, such as basal body temperature or ovulation sticks.
Trial registration number: not applicable

P-470 Sex in the time of Covid-19: Examining the sexual behavior and sexual desire of female adults in Hong Kong

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Study question: How are sexual behavior and sexual desire of Hong Kong women affected during the Covid-19 pandemic?
Summary answer: The Covid-19 pandemic has a negative impact on the sexual life of adult women, in particular, single women who do not have a live-in partner.
What is known already: Since the beginning of the Covid-19 pandemic, there have been ongoing debates on whether lockdown measures would do more harm on individuals or families who are already living in fear of virus infection. Some studies have shown that despite social distancing and measures that limit contact and interaction, women, particularly those who are either married or have a stable partner, were found to be sexually more active and reported stronger emotional bonding with their partners during lockdown. This study attempts to examine any significant changes in sexual behavior and sexual desire of adult females in Hong Kong during the pandemic.
Study design, size, duration: This is a cross-sectional online study examining the sexual behaviors among female adults. The survey was conducted in Hong Kong between July and August 2020, in which the city has been locked down.
Participants/materials, setting, methods: Six hundred and two Chinese female adults (mean age = 32±7.09) were recruited through social media and community networks. Respondents completed the Desire Domain of the Female Sexual Function Index and self-reported frequency of sexual behavior before and during the Covid-19 pandemic. T-tests and ANOVAs were used to compare sexual behavior and sexual desire across demographic groups. Linear regression was conducted with sexual behavior and sexual desire as criterion variable and demographic characteristics as predictors.
Main results and the role of chance: Women reported significantly lower frequency of sexual behavior during the Covid-19 pandemic compared to previously ($t = 8.25$, $P < .001$). Less often did women feel sexual desire or interest during the pandemic ($t = 7.05$, $P < .001$) and a lower degree of sexual desire or interest was reported ($t = 11.16$, $P < .001$). During the pandemic, women who were married or cohabitated reported significantly more frequent sexual behavior than did single women with partners ($P < .01$), while the two groups were comparable in terms of the frequency and intensity of having sexual desire. Linear regression analyses showed a statistically significant reduction in frequency of sexual intercourse during Covid-19 with increasing age ($B = -.19$, $P < .001$), and being single with ($B = -.26$, $P < .001$) or without partner ($B = -.40$, $P < .001$), taking into account all other demographic characteristics. Single women reported significantly less often did they feel sexual desire or interest during Covid-19, while age ($B = -.26$, $P < .001$) and being single without a partner ($B = -.22$, $P < .001$) predicted significantly lower intensity of sexual desire during Covid-19.
Limitations, reasons for caution: Women with either primary or secondary education level are not adequately represented as recruitment was carried out via community network and social media platform, which are more likely to be more accessible by a population who is more tech-savvy and has more access to email.
Wider implications of the findings: We are still in the middle of the pandemic and there is still paucity of data illustrating its impact on sexual life. Current findings could give insight to stakeholders to develop sexual health counselling services that address the negative effect on sexual intimacy arising from sexual behavioral change.
Trial registration number: Not applicable

P-471 Preconception and infertility care across South America: availability of policy, guidelines, recommendations and services

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Study question: What are the available preconception care policies, guidelines, recommendations and services as well as infertility care in South America ?
Summary answer: Preconception recommendations offered by both government and medical societies in South America were fragmented and inconsistent and public fertility care is available in seven countries.
What is known already: Promoting preconception health can potentially improve women's health and pregnancy outcomes. Evidence-based interventions exist to reduce many maternal behaviors and chronic conditions that are associated with adverse pregnancy outcomes such as tobacco use, alcohol use, inadequate folic acid intake, obesity, hypertension, and diabetes. Paternal factors are also influence pregnancy outcomes but male preconcepction health has received little attention so far and he focus remains on women.
Study design, size, duration: Cross-sectional evaluation including ann electronic search and investigation of preconception policy, guidelines, recommendations and services available to healthcare professionals and the general public in south America (N= 11 countries): Argentina, Bolívia, Brasil, Chile, Colômbia, Ecuador, , Paraguay, Peru, Suriname, Uruguay and Venezuela) that took place in June 2020.

No ethical approval was obtained as we used only public avalialble online information.
Participants/materials, setting, methods: Eleven South American countries) were included Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, , Paraguay, Peru, Suriname, Uruguay and Venezuela.we searched Google using advanced search setup for each country with the following words: Preconception care; Pre-pregnancy care; Before pregnancy; Conception; Pregnancy planning; Preconceptual and variations and Policy; Guidelines, Recommendations and services. Data from the Latin American Registry (REDLARA, 2017) and the Latin American Federation of Societies of Obstetrics and Gynecology (FLASOG) was also obtained .
Main results and the role of chance: Government preconcepction care recommendations were available in 10 countries and 11 had family planning guidelines. Seven countries offered either public clinics or financial aid for infertile couples. According to REDLARA 2017 report there are 122 registered centers in South America but the region host much more. The Brazilian registry shows 154 IVF clinics in 2018. Although most countries offered guidance on major issues including folic acid supplementation (n=10), nutrition (n=10), Vaccination (n=11), alcohol consumption (n=11), smoking (n=10), relevant health topics such physical activity (n=7) and obesity (n=7) were left out in 58,3% of the countries. Medical societies provided guidelines on preconcpection care in 7 countries and for other health issues: folic acid supplementation (n=5), nutrition (n=5), Vaccination (n=6), alcohol consumption (n=4), smoking (n=4), physical activity (n=4)and obesity (n=2). When male preconception care was considered, only two countries have public guidelines whereas no medical society provided specific recommendations for men. Sexually transmitted diseases (STD) was another topic of interest for both public (n=10) and medical societies (n=4). STD guidelines were heterogenous and focused more on treatment rather than prevention. FLASOG however displayed guidelines for peconception care and STD prevention.
Limitations, reasons for caution: The search was performed only in Google as it is the most opular search engine. We did not include other search pages asuch as Yahoo and limited our search to the first 3 pages for each search term as people rarely examine more thatn the first there pages.
Wider implications of the findings: Current reccomendations are heterogeneous, fragmented and inconsitent and there is a lack of interest on male reproductive health y. Public fertility care is only available in 7 countries. Collaborative research among countries is necessary so as to develop evidence-based guidelines for preconcepction and fertility care for both men and women.
Trial registration number: not-applicable

P-472 Single mothers by choice - experiences of single women seeking treatment at a public fertility clinic in Denmark: A pilot study

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Study question: Are single women in fertility treatment stigmatised and what do they experience?
Summary answer: The women did not feel stigmatised. They experienced self-blame and negative thoughts about themselves, despite experiencing empowerment and receiving positive reactions from families and friends.
What is known already: Since 2007, medical doctors in Denmark have been permitted to offer medically assisted reproduction (MAR) also to single women. Denmark is a welfare state with a public health care sector providing MAR free of charge, 240 days of paid parental leave, and public full-time day-care. There has been an increase in the number of single women deciding to have children through the use of MAR. These women are referred to as 'single mothers by choice' (SMC), and they have been criticised for being selfish when raising a child without a father. Previous studies have shown how SMC can feel stigmatised. Study design, size, duration: Semi-structured qualitative interviews at a public fertility clinic in Copenhagen, Denmark. Data collection took place between September and October 2020.
Participants/materials, setting, methods: The participants were single and childless women (N=6) undergoing MAR at the Fertility Clinic, Rigshospitalet in Copenhagen, Denmark. Five women received IVF and one received IUI. The women were between 30 and 40 years old and were all residents in the Capital Region of Denmark. The interviews were audiotaped, anonymised, and transcribed in full. Data were analysed using qualitative content analysis.
Main results and the role of chance: Single women did not differ from cohabiting women seeking MAR in relation to their experiences and attitudes towards motherhood. Four main themes were identified; (1) Experiences of single women seeking fertility treatment, (2) Emotions associated with becoming a single mother by choice, (3) The decision of becoming a single mother by choice, and (4) Family formation – a social interaction. The women would have preferred to have a child in a relationship with a partner and the shattered dream about the nuclear family has caused a wide range of experiences and emotions. The women did not feel stigmatised but they all had an awareness of the prejudices other people might have towards single mothers by choice. Hence, they were ready to defend their choice if necessary. On the other hand, they had received positive reactions and the process of becoming a single mother by choice was influenced by their social relations with family and friends. Despite their dream of the nuclear family the women choose to become SMC because motherhood was of such importance and they feared they would otherwise become too old to have children – the biological clock was ticking.
Limitations, reasons for caution: The participants were recruited from a public fertility clinic in the Capital Region of Denmark and may not be representative of all single women seeking MAR. Results might not be transferable to other countries with a different cultural context regarding the societal acceptance of different ways to establish a family.
Wider implications of the findings: This study contributes to the understanding of the experiences of single women seeking fertility treatment in a welfare state where there are no differences in the possibilities for different social classes to seek MAR in the public health care sector.
Trial registration number: N/A

P-473 Should couples be educated on how to try to conceive (TTC) before an infertility diagnosis? A comparative study of fertile, subfertile and infertile couples

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Study question: What sexual strategies do individuals TTC with different fertility status use?; What are the predictors of sexual dysfunction(SD) and frequency of intercourse(IF) when TTC? Summary answer: TTC strategies with no evidence of effectiveness are the most used. Poor marital quality predicted SD, and female SD was a significant predictor of IF.
What is known already: It is well known that couples TTC have low fecundity knowledge. Previous evidence showed that after 12 months the frequency of intercourse decreases. After seeing a fertility specialist couples report a feeling of waiting time by attempting natural conception, which can be associated to evidence of an overestimation and excessive confidence in the success of fertility treatments. Existing guidelines recommend intercourse every other day, but no

comparative studies exist up to date on what sexual strategies are used in different fertility status and what are the predictors of sexual frequency and sexual dysfunction when trying to conceive.
Study design, size, duration: This study is part of a randomized controlled trial on the effects of timed intercourse in psychosocial outcomes. Data was collected between July 2016 and November 2019 via an advertising strategy and obstetrics/gynecology centers. Inclusion criteria were: i) adult in a marital/cohabitation heterosexual relationship >1 year; ii) not knowing of any condition that can prevent pregnancy; iii) being actively TTC; iv) female age >22<42 years old; v) no previous children. Measurements were carried out online.
Participants/materials, setting, methods: Our final sample had 399 subjects (252 women). Participants rated the use of the following strategies: intercourse every other day (EOD), fertile week (FW), basal temperature, cervical mucus monitoring (CMM), ovulation predictor kits (OPK), and keeping legs elevated afterwards (EL). We also accessed psychological adjustment, relationship quality, SD and IF. Comparisons between groups were made by analysis of variance (ANOVA) and Chi-square tests, and logistic regression was used to determine predictors of SD and IF.
Main results and the role of chance: Participants were highly educated (72,8%), in the relationship for 9 years (±5.2), cohabitating for 5 (±3.6), and TTC for 2,5 years (range 0-16). Women were 33 years old (±4.4) and men 36 (±5.5). Regarding fertility status, 22.6% of participants were TTC <12 months, 22.8% >12 months but not diagnosed, 23.6% had a diagnosis but no treatment, and 31.1% had ART. The most reported female strategy in all groups was EL, and the most never used was OPK. Differences were found in EOD, with significantly more women TTC <12 months that never used it, and more women with previous ART using it. Women who had ART are the ones who more frequently use FW and CMM comparing to other women (P>.05). In all groups, the majority reported IF once or twice/week. SD was found in 17.5% of women and 10,9% of men. Age (OR 0.91, 95%CI 0.85-0.97) and SD (OR 2.47, 95%CI 1.02-6.02) were the only predictors of low IF for women, with no significant findings for men. Poor relationship quality increased the risk of SD for both men (OR 0.11, 95%CI 0.03-0.40) and women (OR 0.46, 95%CI 0.03-0.40), and depression increased the risk of female SD (OR 1.24, 95%CI 1.06-0.46).
Limitations, reasons for caution: The cross-sectional nature of this study does not allow causal relationships to be determined. Further cohort studies allowing to assess differences as couples cross different fertility status are warranted. There are important predictors of SD that were not considered, specifically the comorbidity of diseases and pain.
Wider implications of the findings: Findings indicate that individuals TTC are misinformed, and that those using evidence-based sexual strategies are fertility patients. SD should be screened in patients TTC given that it might be an important predictor of IF. Couples might benefit from counselling to improve marital quality and consequently sexual functioning.
Trial registration number: NCT028140069

POSTER VIEWING
PSYCHOLOGY AND COUNSELLING

P-474 Psychosocial aspects of fertility preservation options for young cancer patients in the Czech Republic: a qualitative study

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Study question: What is the real accessibility of fertility preservation techniques and its perception by patients in the Czech Republic?
Summary answer: Fertility preservation options are not offered on a routine basis and often are not part of a treatment plan. Patients wish to be adequately informed.
What is known already: Every year, more than 82.000 people in the Czech Republic develop cancer. The incidence of newly diagnosed cancers in individuals in their reproductive age represents 4.4 % of all newly diagnosed oncological diseases. Because the prognosis of treatment in this group of patients is very favourable, the priority in treatment is the emphasis on quality of life after surviving. One of the important parameters of quality of life for many people is the ability to reproduce and the possibility of having a genetic bond to their children.
Study design, size, duration: This qualitative study was conducted in 2019 and relied on explorative in-depth semi-structured interviews. Participants were chosen through occasional sampling.
Participants/materials, setting, methods: We interviewed 13 cancer patients/survivors in their reproductive age (aged 21 to 36; 7 females and 6 males); 4 close family members of young cancer patients; and 8 experts from relevant professional fields. Data gathered from semi-structured interviews were analysed by interpretative phenomenological analysis.
Main results and the role of chance: Cancer was perceived as a threat to life and one's safety. It causes uncertainty and a feeling of loss of control. It also has a negative impact on a self-concept. Loss of fertility was perceived as a "injury of personality". The possibility of maintaining fertility has increased the subjectively experienced quality of life.

They make decisions under great time pressure, in a mentally demanding situation. They are usually in the early stages of coping with the diagnosis. The main factors that influenced the respondents' decision were the amount and quality of information, psychological stress and time pressure.

Patients and their families are interested in being informed about the risks that the disease and its treatment pose to their reproductive health. They want to be able to decide whether to undergo any of the fertility protection techniques. They want to keep their future open. It is therefore desirable that, within oncofertility, we focus on the process of passing on information and supporting decision-making on issues of fertility and its protection. A tool that could be a good informational platform may be so-called decision aid – a tool used to inform patients about available treatments, along with potential benefits, risks and costs.
Limitations, reasons for caution: As all qualitative data, our findings cannot be generalized. Selection bias could have occurred because it is likely that those interested and open to sharing participated.
Wider implications of the findings: Oncofertility treatment is highly relevant and should be offered and discussed with all patients in their reproductive age. Addressing fertility preservation options should be a part of cancer treatment plan of all these patients.
Trial registration number: 0

P-475 The mediator role of infertility-related psychological inflexibility in the relationship between infertility-stress domains and psychopathological symptoms

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Study question: Does infertility-related psychological inflexibility play a role in the relationship between infertility-related stress domains and psychopathological symptoms (depression, anxiety)?
Summary answer: Infertility-related psychological inflexibility mediated the relationship between infertility-related stress domains and depression. There were no effects between infertility-related stress domains and anxiety symptoms.
What is known already: The emotional impact of infertility may include anxiety and depressive symptoms and these seem to be related to stress. Beliefs about the importance of parenthood (need for parenthood) and rejection of a childfree lifestyle, as well as the impact of infertility in several life areas (social, sexual, and relationship) are conceptually considered two infertility-related stress domains. Although the relationship between infertility-related stress and psychopathological symptoms has been previously recognized, the mechanism underlying this relationship remains undetermined. Psychological inflexibility has been pointed as a core transdiagnostic process contributing to the development and maintenance of several psychological difficulties.
Study design, size, duration: Cross-sectional study. Participants were recruited through the Associação Portuguesa de Fertilidade (patients' association). Inclusion criteria were age (18 years or older) and an infertility medical diagnosis. Data were collected online through self-report instruments between June and December 2019. Participants/materials, setting, methods: A sample comprising 287 women pursuing infertility medical treatment (at different

stages) completed online a sociodemographic questionnaire, the depression and anxiety subscales of the Depression, Anxiety and Stress Scales (DASS – 21), the Psychological Inflexibility Scale - Infertility (PIS-I), and the Fertility Problem Inventory (FPI). Descriptive and correlational analyses were computed through SPSS v. 26, and path analyses were estimated in AMOS (v. 24) with bootstrap procedures (2000 samples).

Main results and the role of chance: Correlation analyses revealed that FPI domains (importance of parenthood and impact on life domains), depressive and anxiety symptoms were significantly and positively associated with PIS-I. A mediation analysis was conducted to examine whether PIS-I mediated the effect of FPI domains on depressive and anxiety symptoms. Paths showing not to be statistically significant were removed. This model showed a good fit to the empirical data: 2(4) = 1.59, p = .810, CMIN/DF = .40; TLI = 1.00; CFI = 1.00; RMSEA = .00, 95% CI = .00 to .06. The effect of the importance of parenthood on depressive symptoms revealed to be both direct (b = .03; SEb = .01; Z = 2.46; p = .014; = .15) and partially mediated by the PIS-I (b = .31, 95% CI = .24 to .37, p = .018). The effect of the impact of infertility in several life areason depressive symptoms was fully mediated by PIS-I (b = .15, 95% CI = .10 to .21, p = .008). This model explained 43% of the total variance of depressive symptoms. No significant effects were found for anxiety symptoms.

Limitations, reasons for caution: Participants were at different stages of their fertility treatment. Data collection was completed online and this tends to recruit participants with more access to online platforms. Results rely on cross-sectional and self-report data.

Wider implications of the findings: Results suggest the relevance of targeting processes encompassing psychological inflexibility, such as cognitive fusion, experiential avoidance, conceptualized self, conceptualized past and future, lack of values clarity, and inability to commit with values-driven actions, in psychological interventions designed for women with infertility.

Trial registration number: N/A.

P-476 Women's attitudes to having children: A mixed-methods study using an online questionnaire of women aged 25-45 years old

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Study question: What are women's attitudes to having children including their ideal age to have children, factors affecting their decision and their understanding of female fertility?

Summary answer: The average age women wanted to have children was age 30, with most still developing their career. They showed a good knowledge of fertility awareness.

What is known already: Women globally are delaying the birth of their first child, with the average age of first birth approaching age 32 in some countries. The fertility rate stands at 1.3 in several European Union countries. Some people are not having the desired family size or are childless by circumstance. We need to ensure we provide fertility education from school-age onwards.

Study design, size, duration: We conducted an anonymous, online survey of multiple choice and open-ended questions using Qualtrics software. The survey was live for 32 days from May 15th, 2020 to June 16th, 2020 and was promoted using social media. A mixed-method approach was used to analyse quantitative and qualitative data.

Participants/materials, setting, methods: A total of 922 women from 44 countries participated in the survey. After filtering out women who did not consent and those who did not want to have children, a total of 834 responses remained. Elimination of blank surveys or insufficient data resulted in a final number of 667 responses.

Main results and the role of chance: The mean age of the respondents was 31.3 (±4.76). The majority were white British (347/667, 52%) and heterosexual (614/667, 92.0%). A high proportion had a university education (195/667, 29%) or postgraduate education (392/667, 59%). The majority were married/in a civil partnership (223/667, 33%) or cohabitating (215/667, 32%). 135/667 (20%) were single and never married.

When asked 'In an ideal world, at what age approximately would you like to have had or have children? a normal distribution was observed with a mean age of 30.2 (±3.2). When asked 'What factors have led you to decide on that particular age?' the most frequent choice was "I am developing my career", followed by "I am not financially ready." Women were asked how informed they felt about fertility. The majority of women said they felt moderately informed (60%, 400/667), very informed (28%, 190/667), or they were not informed at all (12%, 77 /667). Most women thought female fertility decline starts at age 35 (32.8%, 219/667). To the question "What is the oldest age at which women can get pregnant?" almost 70% of women (465/667) believed the oldest age to be between 40-49 and 24%, (160/667) said over 50.

Limitations, reasons for caution: All surveys have a selection bias. The survey was only promoted on social media. As the survey was in English, the women who answered the survey were mainly UK residents who were highly educated.

Wider implications of the findings: In a group of highly educated women, age 30 was the most common age for wanting a child but career development and finances are the main reasons affecting their decision. These women had some understanding of female fertility. Global fertility education is essential to ensure people make informed reproductive choices.

Trial registration number: NA

P-477 The child's right to know vs. the parents' right not to tell: the attitudes of couples undergoing fertility treatments towards identity-release gamete donation

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Study question: Do couples undergoing assisted reproduction treatments (ART) have a different perception of anonymous vs identity-release gamete donation than a population interested in the subject?

Summary answer: Compared with a population interested in the subject, more couples undergoing ART believed the child shouldn't be given information that would identify the gamete-donor.

What is known already: Recent research has investigated the psychological well-being of parents and children born through gamete donation, focusing on the possibility of having the donor's identity revealed. Gamete donors have traditionally been anonymous to recipients and offspring; however, there is a global trend towards programs using donors that are identifiable to the resulting offspring at maturity. While some countries only allow the use of identity-release egg donation, others only allow anonymous-donation, and in some countries both types of donation are practiced. However, the attitudes concerning anonymous vs identity-release gamete donation, in a country where only anonymous donation is allowed, are still unknown.

Study design, size, duration: This cross-sectional study was performed from 01/Sep/2020 to 15/Dec/2020. For that, surveys through online-platforms were conducted, including either patients undergoing ART, (ART-group, n=358) or those interested in the subject, who accessed the website of a university-affiliated IVF-center (interested-group, n=122). Participants in the ART-group were invited via e-mail, with a cover-letter outlining the survey and a link to access it and participants in the interested-group accessed the questionnaire via website.

Participants/materials, setting, methods: The survey collected information on demographic characteristics and the participant's attitudes towards anonymity of gamete donors. The questions were: (i) In the case of children conceived through ART, do you believe that revealing the method of conception may affect the relationship between children and their parents? (ii) Once the method of conception is revealed, do you believe that the child has the right to know the gamete donor? (iii) If yes, when?

Main results and the role of chance: Most of the participants answered that the relationship between children and parents wouldn't be affected by the child's knowledge of the origin of their conception, regardless of the group (83.6% vs 82.7%, for ART-group and interested-group, respectively, p=0.868). Most participants in the ART-group answered that the sperm donor identity

shouldn't be revealed to the child, while only half of the interested-group stated the same (65.4% vs 50.8%, p=0.044). The same result was observed when participants were asked if the oocyte donor should be identifiable (64.8% vs 50.8%, p=0.050). When asked when the donor's identity should be revealed to the child, no significant differences were noted in the responses among the groups (p=0.868). Most of the participants who believe that the child has the right of learning the donor's identity, stated that "the donor's identity should be revealed if the child questions its biological origin" (67.2% vs 67.5%, for ART-group and interested-group, respectively). "Since birth" was the second most common response, (21.0% vs 19.7%, for ART-group and interested-group, respectively), while "when the child turns 18 years-old" (9.2% vs 11.2%, for ART-group and interested-group, respectively), and "sometime during teenage years" (2.5% vs 2.4%, for ART-group and interested-group, respectively) were less common answers.

Limitations, reasons for caution: Lack of adequate opportunities to conduct face to face interview and lack of knowledge of the real state of the website participants, concerning infertility or being involved in ART. The retrospective nature of the study and the small sample size may also be reasons for caution,

Wider implications of the findings: It has been discussed that, whether or not children or parents are harmed by knowing their biological origins, donor offspring have the right to know. However, when facing the situation, couples undergoing ART would argue that in case of gamete donation, there are reasons for not telling the child.

Trial registration number: Not applicable

P-478 Quality of life assessment in women undergoing assisted reproduction. A study of FertiQol and HADS

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Study question: The present study aims to explore infertility-related psychosocial outcomes, including fertility quality of life (QoL), as well as anxiety and depression levels, in women diagnosed with infertility.

Summary answer: Differences on fertility-related QoL appeared when comparing treatment types (gamete donation vs own gamete). Furthermore, statistically significant associations were found between QoL and anxious-depressive symptomatology.

What is known already: Those who wish to have children and do not achieve their objective just like other peers can see their goals and expectations with pessimism, generating concern and a series of negative emotions. Several psychological implications of infertility have been described, such as increased levels of stress, anxiety, depression, decreased self-esteem, mood and hope, or poor relationship adjustment. The emotional impact of infertility in people's life cycle can be so strong that reducing it only to biological aspects would lead to a dangerous situation of neglect. For this reason, QoL assessment in ART becomes an important need.

Study design, size, duration: FertiQol stands as the most widely used tool to assess infertility-related QoL, overcoming the limitations of other instruments that only target specific medical conditions. The present is a multi-site cross-sectional study over patients with infertility (n=104), aiming to explore their fertility-QoL, as well as their anxiety and depression levels, which are symptoms that have been previously associated.Questionnaire administration, and sociodemographic and medical data gathering took place between January 2019 and December 2020.

Participants/materials, setting, methods: Participants were 104 female patients (M.age= 39.8) undergoing or expecting a fertility treatment. The FertiQol Spanish version was administered through mobile app, and its paper version distributed at medical/psychological appointments. QoL was self-reported through FertiQol, assessing the influence of infertility problems in various areas (e.g. impact on self-esteem, emotions, general health, family, partners, social relationships, work, life projects...). Additionally, HADS (Hospital Anxiety and Depression Scale) was provided as a measurement of anxiety and depression levels.

Main results and the role of chance: Regarding treatments, 50.6% of participants were currently undergoing gamete donation while 44.3% were undergoing treatments that involved using their own gametes. After comparing QoL between these treatment types, results showed that patients who underwent egg donation, compared to those who used their own eggs, reported statistically significantly lower scores of QoL in the Social Subscale (p = .03), but not in the other psychological outcomes. Also, statistically significant negative correlations were found between HADS and all core FertiQol subscales (p< .05). Results are consistent with previous studies showing similar associations between fertility QoL and anxiety and depression, as well as with increased psychological negative implications of gamete donation. The majority of participants reported non-pathological scores of anxiety and depression when considering the cut off value of 8 for HADS, thus suggesting the presence of a relatively healthy sample. The number of treatments that patients had previously taken and the years of infertility were not associated with any of the psychological variables.

Limitations, reasons for caution: Some limitations to consider are presence of co-morbid diagnosis, differences in medication, or patient's cultural backgrounds.Also, conclusions should be interpreted cautiously since the design doesn't allow causal inferences. Further investigations should consider a continuous assessment to explore changes in psychological well-being at different points of intervention, specially with gamete donation.

Wider implications of the findings: The great advantage we've seen so far when using FertiQol is the possibility to identify more accurately the true impact on other aspects of patient's well-being besides the emotional area.ART professionals, including psychologists and counselors,will have more information within a small amount of time about QoL when using this tool.

Trial registration number: 1503-BCN-019-DG

P-479 Are FET and IUI cycles less emotionally difficult for patients than IVF? Evidence from smartphone app based emotional tracking data

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Study question: Is the emotional experience different in FET and stimulated IUI cycles compared to IVF cycles?

Summary answer: Emotional tracking data demonstrated cautious optimism and lower harm emotions in IUI, but FET cycles are associated with higher harm emotions than fresh IVF.

What is known already: It is sometimes claimed on clinic websites and by advocates for elective freeze all that FET cycles are inherently less stressful. However, little research evaluates the emotional difference between fresh and frozen cycles and the assumed emotional ease of FET may reflect clinician interpretation/bias rather than patient's lived experiences. Many undertaking FET will have experienced disappointment in a fresh cycle and with increasing cycles comes increased cost. IUI treatment is perceived as less physically and emotionally intense, but studies have shown increased depression levels after a failed IUI cycle and high drop-out.

Study design, size, duration: Retrospective single-centre analysis of anonymised emotional tracking data entered by 707 patients using MediEmo app alongside IVF, 104 during stimulated IUI and 65 during medicated FET from May 2017-September 2020.

MediEmo includes medication timeline/ notifications, coping tools and emotional tracking. Patients rate 2 questions daily in each emotion domain (challenge, threat, harm, e.g. 'I am feeling tense') on a 0-3 scale and indicate coping ability ('I am unable to cope with the stress I'm experiencing').

Participants/materials, setting, methods: Egg donor, recipient and fertility preservation cycles were excluded. Mean and standard deviation of scores in each mood domain entered per cycle day were calculated, centred on luteal day 0/ egg collection, from cycle day +/-14.

Between group analysis performed using one-way analysis of variance (ANOVA) is presented here. Time series analysis, graphical presentation of emotions by cycle day and analysis of cycles resulting in live birth or return for further treatment will be presented.

Main results and the role of chance: Analysis of emotional tracking data demonstrated patients experience higher levels of positive challenge emotions (confident/encouraged/hopeful/positive) during FET and IUI cycles than fresh IVF: mean(s.d) score FET 1.64(1.1), IUI 1.74(0.89), IVF 1.48(1.06) (ANOVA p<0.00001). The difference between FET and IUI challenge levels was not significant (p=0.07).

Threat emotions (worried/nervous/anxious/tense) are significantly lower in FET compared to IVF and IUI cycles: FET mean 0.67(0.91), IUI 0.97(0.90), IVF 0.87(0.91), (ANOVA p<0.00001). The difference between IVF and IUI threat levels was not significant (p=0.06).

However, the harm emotions (sad/discouraged/disappointed) experienced by patients are significantly higher in FET, mean 0.62(0.89) compared to IVF 0.50(0.81), which are higher than IUI cycles, 0.36(0.68), (ANOVA p<0.00001). There were no significant differences in numbers recording intolerable stress between the three groups (FET mean scores 0.24(0.66), IUI 0.21(0.58), IVF 0.21(0.59), (ANOVA p =0.67).

As this is retrospective observational data, there are differences between groups in addition to treatment modality, e.g. mean patient ages in the FET and IUI groups were older than those entering data during IVF; FET 34.2(4.09), IUI 33.9(5.2), IVF 32.6(4.47). However, age was not correlated with levels of challenge emotions, suggesting assumptions that patient emotions, e.g. hopefulness, are closely linked to objective prognosis may be flawed.

Limitations, reasons for caution: Emotional data was only available for those who chose to use MediEmo, entered emotional tracking data and who gave consent for use of data in research. As such, this analysis may not fully reflect all patients' experiences. However, these limitations apply to all groups and should not prevent useful comparison.

Wider implications of the findings: Patients have less contact with clinic staff during FET or IUI than fresh IVF cycles. Fertility staff need to ensure availability of support during all treatment cycles and be empathic, particularly for those embarking on FET, who may still be coming to terms with a failed fresh transfer cycle.

Trial registration number: Not applicable

P-480 Adding fuel to the flame of low fertility: Fertility intention and perceived socio-political stability of young adults in Hong Kong

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Study question: How does perceived socio-political stability impact on the fertility intention of Hong Kong adults?

Summary answer: Political and economic uncertainties play an especially significant role in reproductive decision-making among young adults in Hong Kong, where traditional family beliefs diminish in importance.

What is known already: Hong Kong has one of the lowest fertility rates in the world, despite the importance placed on values like family lineage and childbearing as a filial obligation. Previous investigation of Hong Kong students' perception of reproduction showed that proximal factors such as having a stable relationship and personal maturity as the most important conditions for parenthood. It is yet to be explored whether more distal factors such as the economy and political stability also play a role in reproductive decision-making among Hong Kong adults, especially under the influence of the Anti-Extradition Bill Movement from onwards.

Study design, size, duration: This study uses cross-sectional data from an online survey that explores the fertility attitudes, intentions, and behaviours and perceived socio-political stability of Hong Kong Chinese adults. Data were collected between July and August 2020.

Participants/materials, setting, methods: Participants were 629 childless Hong Kong Chinese women (mean age = 30±6.68) recruited through community network and social media. Participants answered questions on fertility intention, and rated the extent to which 'political environment', 'economic stability' and 'education system' are important social-political factors in considering family formation, and to which they agree with traditional family beliefs. We conducted binary logistic regression with fertility intention as the criterion variable and social-political factors of family formation as predictors.

Main results and the role of chance: Participants considered the 'political environment' and 'education system' very important factors when considering family formation, especially among those aged 25 or below. More than 70% of respondents said they would like to have children, yet only 44% said they plan on actualizing their parenthood goals in the near future. Regression analyses showed significant main effects of age and gender on fertility intention, such that younger (P<.001) and male (P<.01) participants were less likely to intend on becoming parents. The more participants valued 'political environment' (B=0.48, P<.001) and 'economic stability' (B=0.39, P<.05), the less likely it is for them to intend on becoming parents, controlling for age and gender. There was also significant interaction between age and importance of 'political environment' (P<.01), indicating that for whom 'political environment' is an important condition for parenthood, younger participants had lower intention of having children than older participants. There was no significant effect of gender. Overall, participants did not subscribe to traditional beliefs such as that childbearing is 'a necessary part of married life' or that it is 'a filial obligation as sons or daughters' (ratings = 1.95 – 3.05, out of 5).

Limitations, reasons for caution: Participants were recruited by self-selection through community network and social media, potentially favouring individuals who were more concerned with fertility issues to begin with. Additionally, men were largely under-represented in this sample (15%), potentially obscuring any significant gender differences relating to traditional family beliefs and determinants of reproductive decisions.

Wider implications of the findings: With economic and political uncertainties expected to persist, these findings call for increased psychosocial and fertility education for young adults in navigating long-term parenthood goals and reproductive options, and policies that assist young adults in overcoming personal and structural barriers to parenthood amid diminishing confidence in governmental support.

Trial registration number: not applicable

P-481 COVID-19 pandemic: the emotional impact comparing men and women on assisted reproductive treatment

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Study question: How has the COVID-19 pandemic affected the psychological aspects of men and women undergoing reproductive treatments?

Summary answer: The women were more emotionally affected due to the COVID-19 pandemic than men, especially increasing anxiety and fear of not achieving pregnancy.

What is known already: COVID-19 pandemic required changes in behavior and plans of most people worldwide, including patients undergoing assisted reproductive treatment (ART). The reproductive societies recommended immediate cessation of all new fertility treatment cycles, arousing different opinions from patients and providers, concerned that a delay of months may affect clinical outcomes. The fear, social distancing and financial insecurity are enough reasons for worry and anguish, and the uncertainty of resuming plans of parenthood make the scenery even more challenging. Therefore, the psychosocial aspects' evaluation of these patients during the pandemic is fundamental for better comprehension, management, and reception in this especially challenging moment.

Study design, size, duration: Cross-sectional study using data from a centre of reproductive medicine between June and August 2020. The sample was composed of 120 patients (54 men and 66 women), 14.16% undergoing frozen embryo transfer (FET), 77.5% in vitro fertilization (FIV), 1.6% semen freezing collection and 6.6% oocyte freezing.

Participants/materials, setting, methods: The data were extracted from an electronic questionnaire elaborated by the clinical team, which included questions about the patients' psychological aspects, applied one day before ART. The answers were compared between men and women,and between the types of ART used by each patient. The statistical analysis was made using the program SPSS for Windows. The Chi-Square test was used to compare the study groups, considering p<0.05 statistically significant.

Main results and the role of chance: It was observed that 23/54 (42%) of men and 42/66 (63%) of women were at least partially emotionally affected by the pandemic (p=0.027). Comparing feelings between groups (men and women, respectively) the following results were observed: optimistic (42,1% vs 57,9% p=0.664), hopeful (32,3% vs 67,7%, p=0.098), anxious (22,6% vs 77,4%, p=0.004), calm (60,7% vs 39,3%, p<0.001). Also, 27% of men and 39.3% of women felt more anguished than normal, which was mostly expressed through anxiety (36.7% vs 63.3%, p=0.113), followed by irritability (54.5% vs 45.5%, p=0.421), eating habit change (42.5% vs 56.5, p=0.962) and sleep disorders (28.6% vs 56.5%, p=0.215). Most patients (96.6%) reported having

somebody to share their feelings and didn't want to be contacted by the clinic's psychologist (92.5%); 26.3% of couples had their relationship positively affected. Comparing feelings between patients undergoing FET vs FIV, respectively, were found: optimistic (47.0% vs 29.0%, p=0.142) and anxious (23,5% vs 27.9%, p=0.70).

Limitations, reasons for caution: The data was collected at one point, in the worst moment of the pandemic in Brazil, which may have influenced some of the answers. The small sample size is due to the lower number of procedures in this period.

Wider implications of the findings: The feelings were similar between groups. However, women seemed to be more fearful of not being able to realize the parenthood dream. Anxiety was the main symptom in both groups, being more prevalent in women. This study reinforces the importance of having mental health professionals in assisted reproductive clinic.

Trial registration number: not applicable

P-482 According to donor conceived adults, continuing the sharing-information process with parents about the donor conception is easier when the father took part in disclosure

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Study question: The opinion and feelings of adults after disclosure of the use of donated gametes for their conception

Summary answer: Disclosure is beneficial for 85.1% of donor conceived participants. Continuing the sharing-information process with parents is significantly easier when the father took part in disclosure

What is known already: Sharing information about the use of donor-conception with offspring is a complex process at several levels, involving in particular the parents' will, the circumstances of disclosure, the child's reaction, or the age of the child at disclosure. In this process, the child has a central position, source of force or friction. However, little is known about the opinion and feelings of adults who have been conceived through gamete donation.

Study design, size, duration: An online survey between March 2019 and September 2020. The opening of investigation was announced in media (press, radio, television), social networks, professional websites (CECOS French Federation…) and through interest groups (PMAnonyme, BAMP!, MAIA, ADEDD…) in France.

Participants/materials, setting, methods: Participants completed a standardized questionnaire intended for (spermatozoa or oocyte) donor conceived adults, available on the AP-HM website

Main results and the role of chance: 114 participants responded to the survey, 14 men and 100 women. The average age is 32.9 +/- 7.35 years old. Among them, 111 (97.4%) are born using sperm donation, 2 (1.8%) using oocyte donation, and 1 (0.9%) using double gamete donation. Their parents are 110 heterosexual couples, 3 single mothers, and 1 lesbian couple. For 113 (99.1%) of them, the parents had ART in France. Disclosure took place when they had 18.34+/-11.7 years old. The average time between disclosure and the survey participation is 14.58 +/- 8.77 years. Information was transmitted by the mother for 47.4%, the father for 8.8%, by both parents for 29.8%, and others for 14%.

The circumstances of information are: always knew it (11.4%), at a time chosen by the parents (36%), following a health event (7%), during a conflict (16%), following my questions (14%), by chance discovery (13.2%). A subsequent sharing process was possible after disclosure for 89 (78.1%) participants, and impossible for 25 (21.9%) of them. The sharing process is considered as not difficult for 49.5%, but difficult for 50.5%. It is significantly easier to repeat discussion about the donor conception with their parents when the father took part in disclosure (p = 0.02).

Limitations, reasons for caution: Most of the participants are members of interest groups. This may induce a risk of selection bias. Participants are primarily conceived using donated spermatozoa within heterosexual couples. This conclusion may not be applied to oocyte donation or other family models.

Wider implications of the findings: The present findings highlight the role of the father at the disclosure step, so that the subsequent information-sharing process is easier within the family.

Trial registration number: Not applicable

P-483 "It's a bigger deal for her": Understanding differences in partner involvement in reproductive health decision-making

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Study question: What is the level of partners involvement in family-building and reproductive health decisions?

Summary answer: Level of Involvement ranges from active decision-makers and equal-partnerships to indifferent or no partners. Fertility education needs to be tailored according to level of involvement.

What is known already: Partner involvement is very important in alleviating stress associated with fertility and reproductive health decisions. Recent global health policies have recognised the importance of improving knowledge and awareness of fertility and reproductive health among couples, additionally there has been a concerted effort among reproductive health groups, to improve fertility awareness. Understanding the role partners play in decision-making is therefore important in order to ensure that men and women achieve their family building intentions. In this study, we interviewed men and women, to understand partners involvement in decision-making.

Study design, size, duration: The study was a qualitative component of a wider mixed methods study. We carried out 35 in-depth interviews with 15 men and 20 women. Interviewees were purposively sampled to include men and women from the reproductive age range (18-45 years) and of varying ethnic and educational backgrounds.

Participants/materials, setting, methods: Interviewees were sampled from a UK cross-sectional survey on Fertility Awareness. Survey participants were recruited nationwide via online newspaper and social media adverts and of those who agreed to a follow-up interview, 35 were included this study. Interviews lasted an hour on average. Data was transcribed and analysed via framework analysis. Favourable ethical opinion was given by University College London Research Ethics Committee.

Main results and the role of chance: We identified four kinds of partner involvement and impact, as follows:

Drivers: These are **active decision-makers** who play a bigger role. The decision is usually clear and directive and are typically women. Quotes describing drives include: "Her body her rights", "I just went with her [views]", MP5 - Male, Age 38. "She carries the pregnancy, and it's a bigger deal for her so it's important for her to choose." MP1 - Male, Age 45.

Sharers: In these **equal partnerships,** joint decisions are important. Being similarly minded and aligned is key to achieving desired family building decisions. . "It was very mutual because he'd actually been talking about it for a long time… so we were both completely ready." Female, Age 31.

Neutrals: general **indifference** to family-building decision-making and are not as proactive as the drivers.

Solo: includes individuals with no partners or those who haven't met a suitable partner at the right time or until later in life, or those for whom singleness by choice is key to their decision-making. "If I did meet the right person yeah, I would love one more child, because I've always wanted two" Female, Age 36.

Limitations, reasons for caution: One of the main methodological limitations of this study is that the interviewees were self-selected, which has implications for generalisability. The results necessarily reflect the views of those who were willing to participate. Online recruitment method could result in potential bias towards respondents of higher socioeconomic status.

Wider implications of the findings: To improve fertility awareness, current initiatives need to further explore the impact of partners in family-building decision-making in order to have effective campaigns which can help men and women achieve their desired fertility intentions.

Trial registration number: Not applicable

P-484 Progesterone supplementation in modified natural frozen embryo transfer (mNC-FET) does not cause mental health adverse effects - A sub-study of a multicenter RCT

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Study question: Do women undergoing mNC-FET with progesterone supplementation experience mental health adverse effects at a greater rate compared to a control group.
Summary answer: Progesterone supplementation does not affect mental wellbeing in women undergoing mNC-FET.
What is known already: Women and men undergoing assisted reproductive treatment more likely to experience stress and other adverse psychological effects than the background population. Various factors such as parental age, cause of infertility and treatment method have been shown to affect patient well-being. Progesterone supplementation is known to cause various physical adverse effects, yet few studies have investigated the potential mental health adverse effects of progesterone supplementation in FET.
Study design, size, duration: This is a sub-study of an ongoing RCT investigating the effect of luteal phase progesterone supplementation in mNC-FET. The aim is to investigate possible mental health adverse effects of progesterone. From 2019-2021 a total of 164 women were included (n=84 and n=82 in the progesterone and control group, respectively). The health and wellbeing self-reporting survey was fulfilled after randomization on hCG trigger + 11 days.
Participants/materials, setting, methods: A validated, electronic questionnaire in Danish was used to measure mental wellbeing in women aged 18-41 years undergoing mNC-FET with and without use of progesterone supplementation in the luteal phase at seven Danish public hospitals. Women were randomized to either progesterone treatment or no progesterone by a computerized randomization algorithm with minimization for female age >=37 years, previous oocyte retrievals and previous FET. Comparisons of survey responses were performed by chi-square tests.
Main results and the role of chance: The survey response rate was 68%. We observed no significant differences in any of the three items between the progesterone group and the control group. On the first item "to which degree have you felt sensitive due to treatment", 56% and 52% responded "to a large degree" or "to some degree" sensitive in the progesterone vs. control group, while 25% and 34% vs. 19% and 13% responded "to a lesser extent" or "not at all" sensitive in progesterone vs. controls (P=0.35).

On the second item, "to which degree have you felt aggressive due to treatment", 10% and 9% responded "to a large degree" or "to some degree", 29% and 22% answered "to a lesser degree" and 62% and 70% responded "not at all" in the progesterone vs control group (P=0.57).

On the third item "to which degree have you cried unexpectedly due to treatment" 25% and 18% responded "to a large degree" or "to some degree" in the progesterone vs control group, 20% and 27 % answered "to a lesser extent", while 55% in both groups answered "not at all" (P=0.44).
Limitations, reasons for caution: In a self-reported survey selection bias, due to a less than 100% response rate, and reporting bias cannot be excluded. However with the possibility to answer the survey online at leisure, the risk of reporting bias is minimized.
Wider implications of the findings: A large concern for clinicians working with ART is patient wellbeing. Our study suggests that luteal phase support does not cause extra emotional distress, though further research is needed.

Trial registration number: NCT03795220

P-485 A systematic analysis of acupuncture for IVF treatment: how should the HFEA traffic light scale for add-ons rate it?

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Study question: How should acupuncture be rated on the Human Fertilisation and Embryology Authority traffic light scheme for IVF add-ons?
Summary answer: Randomised controlled trials examining the possible effects of acupuncture on IVF success rates are conflicting, and acupuncture should be rated amber.
What is known already: The use of complementary therapies in assisted reproduction and IVF has become increasingly more commonplace in recent years. Patients seeking to maximise their chances of conception are often interested in purchasing additional treatments (termed 'add-ons') to augment their treatment cycle, often at a high price even in the absence of robust underlying evidence. The use of acupuncture is popular due to putative holistic benefits including stress reduction, and the perceived lack of side-effects and minimal invasiveness. The HFEA traffic light system has not yet rated any complementary therapies, even though these are promoted by fertility clinics.
Study design, size, duration: A systematic review of randomised controlled trials (RCTs) of acupuncture during IVF treatment was conducted. A literature search for acupuncture studies was conducted on the PubMed database and the University College London (UCL) library database. Search terms used were "acupuncture" paired with "IVF", "*in vitro* fertilisation", "assisted reproduction" and "RCT". Study quality and variance in treatment protocols were assessed, to understand both any evidence and its quality. Statistical analysis was performed using STATA.
Participants/materials, setting, methods: The UCL library database yielded 403 individual search results and PubMed database yielded 47. Papers were screened and sorted according to the inclusion and exclusion criteria. Inclusion: publication in English, in an English-language journal; RCT; intervention administered during IVF; either pregnancy rate (PR), ongoing/clinical PR or live birth rate (LBR) reported. Exclusion: reviews; not in English; not RCT; above outcomes not reported.
Main results and the role of chance: After final screening, a total of 34 acupuncture RCTs were included in the review and meta-analysis. The sample sizes of the studies analysed ranged from 44 to 809 (median 162). Only a minority of studies (18%, 6/34) involved blinding of both assessor and participant, while foregoing incorporation of blinding into study design was most common (44%, 15/34 studies). There was little consistency regarding the timing of acupuncture treatment during the IVF protocol across RCTs. A total of 21/34 studies (62%) had a protocol involving acupuncture administration before and after the embryo transfer procedure on the day of transfer. The number of needle insertions during the treatment protocols ranged from 5-13 (mean 8.7). Manual acupuncture only was performed in 8/34 (24%) of studies and 26/34 (76%) utilised electrical stimulation of at least some of the acupuncture needles. Out of 34 RCTs, only 10/34 studies (29%) reported LBR. The meta-analysis included all identified RCTs. The most clinically relevant outcome measure reported in each study found a slight benefit of acupuncture for overall IVF success (OR 1.37, 95% CI 1.13-1.65) however the effect was diluted when only comparing studies reporting LBR (OR 1.14 ,95% CI 0.81-1.61).
Limitations, reasons for caution: Methodological heterogeneity of acupuncture RCTs in IVF (needling location, stimulation, retention time, repetition and timing) complicates data pooling. Underlying neurophysiological mechanisms of action are still being clarified and may help delineate optimal regimens, potentially tailored to individual causes of infertility. Treatment safety and potential for worse outcomes must be considered.
Wider implications of the findings: Complementary therapies are a popular add-on for IVF treatment but assessing them from a robust biomedical perspective is challenging due to issues with study design (including controls), study quality and general attitudes. For acupuncture, future research should arguably focus on biomedical perspectives and shift away from Traditional Chinese Medicine philosophies.

Trial registration number: Not applicable

P-486 Psychological determinants of the decision to attend couples infertility counselling

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Study question: May specific psychological variables related to the experience of infertility have a predicting effect over the decision of accepting counselling?
Summary answer: Specific infertility related sources of sufferance including low levels of infertility self-efficacy and poor quality of life significantly predict the request for professional help.
What is known already: Available data on the access to infertility counselling services suggest that only 10-34% of patients who are offered such opportunity actually pursue it. Qualitative studies pointed out that this might be due to a lack of information about available support and to negative attitudes toward counselling. It seems also that women and men who accept counselling have worse levels of psychological distress. However, there is a lack of quantitative studies on the topic and among those available none used measures that are specific to the experience of infertility itself
Study design, size, duration: The data presented herein are part of a larger data collection promoted by the Italian Ministry of Health on the psychological impact of assisted reproduction. The present study is a cross-sectional research and involves a sample of 184 patients, composed by 92 women waiting for infertility treatment and their partners, enrolled between October 2019 and October 2020 at the Infertility and IVF Unit of the S. Orsola University Hospital in Bologna, Italy.
Participants/materials, setting, methods: Participants were voluntarily enrolled in the study at their first medical consult. They were informed about the possibility to attend free couples infertility counselling sessions and asked to fill in the following questionnaires: Infertility Self-Efficacy Scale (ISE); Fertility Quality of Life (FERTIQoL); Dyadic Adjustment Scale (DAS). To attend infertility counselling a shared agreement between partners was requested. Couples who agreed to the study but not to counselling sessions were provided only with questionnaires.
Main results and the role of chance: The 34.8% (n = 32 couples) of the sample accepted to receive counselling sessions. The two groups (counselling vs no-counselling) were comparable in all socio-demographic variables, aside for education, with higher education levels in the counselling group.

Overall, the counselling group reported greater psychological sufferance than the no-counselling group, with lower scores at the ISE, FERTIQoL, and DAS questionnaires.

Regarding which factors predicted the decision to attend counselling sessions, logistic regression analysis showed that: for the female partner's dimensions low scores at the ISE and at the Emotional subscale of the FertiQoL were predictive of accepting counselling (when scores increased the odds of being in the counselling group would decrease by 46% and 8% respectively); for the male's partner dimensions, predictive factors were low scores on the Social subscale and high scores on the Relational subscale of the FertiQoL (when scores increased the odds of being in the counselling group would decrease by 8% and increase by 10% respectively).

In conclusion, impairments in self-efficacy, emotional well-being and social life may drive a greater need for help, but a close relationship with the partner may be also necessary to predispose men to accept couples infertility counselling.
Limitations, reasons for caution: Data were collected from a well enough homogeneous sample which may have helped in better enhancing the specificity of infertile couples' needs. However, the small sample size and the fact that data were collected from a sole Italian clinic may impact the representativity of our results.
Wider implications of the findings: Findings provide important information for clinical interventions with infertile couples. Patients accepting counselling might be having a worse adjustment to the experience of infertility. Besides, women and men may be affected in different ways. Men's closeness to the partner might be a favourable factor and should be further studied.

Trial registration number: The study was approved by the Ethical Committee of the S. Orsola Hospital, University of Bologna (CE: 273/2018/Sper/AOUBO) and funded by the Italian Ministry of Health (J33C17000560001)

P-487 Couples undergoing first level assisted reproductive techniques: An Actor-Partner interdependence model of dyadic adjustment, psychological symptoms, alexithymia and romantic attachment on body-image avoidance

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Study question: The study aims to assess the commonalities and interdependence of couples undergoing first-level Assisted Reproductive Techniques (ART) as regards body-image avoidance referred to body-image dissatisfaction.
Summary answer: Partners' functioning seem specular yet not interdependent, as not showing a couple-as-a-unit modality of functioning. Body-image avoidance is only influenced by intra-personal variables. What is known already: Stressful bodily emotions and body perception related to infertility and ART are critical aspects for people desiring having children. Infertility undermines women's self-esteem and body-image, damaging their self-identity as women, while in males infertility associates with body dissatisfaction referring to perceived reduced physical fitness and personal failure, thus undermining their body virility. For infertile women, body-image dissatisfaction associates with reduced marital adjustment, and *vice-versa;* yet no study has considered how males body-image dissatisfaction associates with marital satisfaction. Nonetheless, couples should be considered as a unit, considering that infertile couples' adjustment is influenced by their own, and their partners', perceived stress.
Study design, size, duration: The study follows a cross-sectional design and is part of an ongoing transversal and longitudinal project, started in 2012, investigating the well-being of couples undergoing ART. For the present study only a minority of the existing data were considered, thus only including couples at the first level of ART with the intent of investigating couples' commonalities and interdependence before treatments pervasiveness increases.
Participants/materials, setting, methods: Minimum N=79 couples needed to be considered according to Power analysis results. N=118 couples aged 24 to 46 years (women Mage=34.92, SD=3.98; men Mage=37.45, SD=5.25) were included, and declaring trying to get pregnant from 1 to 8 years (M= 3.18; SD=1.99) and to never had children, although 22.9% of women had at least an abortion. Participants completed the Body-Image Avoidance Questionnaire, Toronto Alexithymia Scale-20, Dyadic Adjustment Scale, Symptom Checklist-90-Revised and Experiences in Close Relationships Scale-Revised.
Main results and the role of chance: The sample presents non-clinical levels of functioning referring to their psychological symptoms, alexithymia and body-image avoidance. Multivariate rank tests show that females report significantly higher levels of body-image avoidance (stat=-5.73; *adj.p*=.001), psychological symptoms (stat=-4.58; *adj.p*=.001) and romantic anxious attachment (stat=-3.33; *adj.p*=.005). These differences were confirmed also after applying multiplicity control. Moreover, bi-variate Pearson's r correlations show an association among partners' dyadic adjustment (r=.293; $p<.001$), albeit their overall level of dyadic adjustment is low. Significant correlations among partners also emerged as regards psychological symptoms (r=.258; $p<.001$) and alexithymia (r=.16; $p=.05$). The couple-effect, thus considering the couple as the unit of analysis, was modeled through an Actor-Partner Interdependence Model. For both partners, dyadic adjustment's actor-effect associates with body-image avoidance (women: $\beta=0.133$, $p=.026$; man: $\beta=0.133$, $p=.026$). Furthermore, for both men and women, psychological symptoms' actor-effect associate to body-image avoidance (women: $\beta=0.467$, $p<.00$; men: $\beta=0.499$, $p=.001$). Comparing the level of influence of actor and partner effects among partners, the psychological symptoms' actor effect results significantly more influential than the partner-effect (women: $\Delta=0.378$, $p=.015$; men: $\Delta=0.587$, $p=.001$). Only for males, alexithymia's actor effect is significant ($\beta=0.499$; $p=.001$).
Limitations, reasons for caution: Results should be considered in light of some limitations. Specifically, the cross-sectional study design, lack of a control

group with no infertility issues, the use of self-report measures, homogeneity among couples and the sample small sample size (although sample size was appropriate to retain a power of at least .8).
Wider implications of the findings: Results support the differentiation of gender-specific psychosocial interventions along the ART path, preventing and mitigating the negative impact of infertility and ART on body-image dissatisfaction and avoidance and on the couples' well-being.
Trial registration number: Not Applicable

P-488 Patients' attitudes towards the anonymity of gamete donation in Spain

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Study question: To what extent do infertility patients in Spain support different forms of anonymity for oocyte and sperm donation?
Summary answer: Most patients who undergo treatment with donated gametes in Spain consider that their children should not know the identity of the donors.
What is known already: Spain has a large tradition of gamete donation, probably influenced by its law that requires gamete donation to be anonymous for the donor and the recipient. Although there is a growing support for openness and identity-release in gamete donation, Spanish Society of Fertility has generated an Anonymity in Donations Framework Document which recommends revelation to the donor-conceived children their biological origin preserving the identity. However, there is no information on what the preferences of the patients are regarding the disclosure of the origins to their children.
Study design, size, duration: A prospective, cross-sectional multicenter study that includes all eleven clinics in Spain and involves women who had used donated gametes. From September to October 2020, a self-administered questionnaire was sent out to a total of 57 women which were asked to indicate their responses on a 7-point Likert. 57 (100%) women anonymously completed the questionnaire.
Participants/materials, setting, methods: The participants were asking for their socio-demographic characteristics, their opinions concerning secrecy or disclosure of the method of conception towards the child, what type of information should the child have access to – identifying or non-identifying – and whether they intend to inform their child and relatives about his/her origin. Statistical analysis was performed with Chi square test for dichotomous variables and one-sample T-Student for Likert items. p <0.05 was considered significant.
Main results and the role of chance: Unlike homosexual or single women, 60% of heterosexual couples refuse to inform their offspring about the origin of their gametes and 47,5% would not tell anyone.

Patients do not want to know the identity of donors (0,276 p 0,001) and they consider that knowledge about the origin of the gametes (0,278 p 0,001) or the identity of the donor (0,178 p 0,001) is not important to a child.

Patients do not believe that the donor has the right to know the identity of the offspring (0.098 p <0.001) but they agree with his/her right to anonymity (0.679).
Limitations, reasons for caution: While the multicenter study design and the extraction of a complete time series from the population under study strengthens validity, the study is limited to women, without being able to extrapolate the results to men or children born by gamete donation, which constitutes a limitation.
Wider implications of the findings: The findings of this study can be used as a basis for further discussion between regulators and professionals with respect to anonymity related to donor conception. These opinions should be considered carefully in legal and ethical discussions on gamete donation.
Trial registration number: Not applicable

P-489 Stress scale and coping strategies adopted by Brazilian ART patients during COVID-19 outbreak: attention need for young, women, first IVF attempt and compromised income patients

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Study question: What are the stress scale and coping strategies of patients who were unable to start/continue an IVF cycle due to COVID-19 interruption on ART treatments?
Summary answer: Stress scale was associated to specific coping strategies and was higher for women, patients in first IVF treatment, had compromised income and younger than 38yo.
What is known already: In March 2020, due to the COVID-19 outbreak caused by the SARS-CoV-2 virus, human reproduction societies, have recommended discontinuation of reproductive care, except for the most urgent cases. After few months, the treatments were resumed following proper safety guidelines. Infertility diagnostic and treatments are severe stressors, causing anxiety, depression and general emotional distress. The disruption of treatments and the pandemic uncertain scenario in all life aspects, certainly have a great impact on mental health of ART patients. There is an urge need to assess the level of stress and coping strategies in this population to offer suitable support and care.
Study design, size, duration: Prospective, Brazilian multicentric study (6 clinics of ART located in São Paulo, Campinas, Belo Horizonte and Brasilia), with the application of an anonymous online survey of stress scale and 14 coping strategies to 1500 patients (male and female) that had their treatments interrupted or unable to start during the months of March, April and May 2020. The online survey was sent during the months of August/September and responses were collected until early October/2020.
Participants/materials, setting, methods: The stress scale level was assessed using the Perceived Stress Scale protocol (PSS), and coping strategies using the Brief COPE scale protocol. Social-demographic variables (gender, age, city of residence, marital status, time of infertility, previous IVF treatments and financial impact) were included in the survey. Marginal statistical analyses were performed accordingly (t test, Mann-Whitney, Kruskal-Wallis, chi-square test) and a linear regression model was carried out to calculate the effect of COPE strategies on stress scale.
Main results and the role of chance: Survey's response rate was 44.4% (n=666). The majority were women (83.3%), married (93.2%, mean of 9,41±4,76 years), deal with infertility for 2-5 years (51.5%), had done a previous IVF treatment (61.4%, mean of 2,33 treatments) and had a work activity (83.9%). Almost 40% had their income compromised by the pandemic. Mean age was 38.47±4.99 years (≥38 yo=59%). Stress scale was higher for women (p<0.0001), patients that were in their first IVF treatment (p=0.011), had their income compromised (p=0.001) and were younger than 38yo (p<0.0001). The most frequent coping strategies (score 5-8) were planning (87.7%), active coping (83%), positive reframing (72.1%) and religion (71.7%). Women used more emotional support, religion, venting (all p<0.0001) and self-distraction (p=0.002) as coping strategies than men. Younger patients (<38yo) reported more use of substances (p=0.002) and self-distraction (p=0.001) than older patients. Lower income was associated with denial (p=0.002) and less use of religion (p<0.0001) and patients that were about to start their 1st treatment used more venting (p<0.0001) and denial (p=0.003) than recurrent patients. The linear regression analysis showed that higher stress was associated to planning, religion, self-blame, venting, self-distraction and behavioral disengagement and lower stress scale to active coping, emotional support, positive reframing and acceptance.

Limitations, reasons for caution: This study was performed in Brazil, one of the most affected countries by the COVID-19 outbreak, which may limit the generalizability of the findings. Another limitation was the impossibility to compare the stress scale and coping strategies findings in this population prior to the pandemic.
Wider implications of the findings: Being a woman and have a compromised income were expected stressor factors. Surprisingly, first IVF attempt and younger patients showed higher stress scale and the use of psychological defense mechanisms, such as the use of substances, denial and self-distraction. Continuous emotional support should be offer for all ART patients.
Trial registration number: Not Applicable

P-490 Recurrent pregnancy loss acts as a posttraumatic stress event in both women and men

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Study question: What are the psychological impacts of recurrent pregnancy loss (RPL) on men and women and their interdependencies?
Summary answer: Women show higher psychological risks than men, except for lack of social support. Avoidance behaviour of men correlates with higher posttraumatic stress of their partner.
What is known already: About 1-3% of all couples trying to conceive are affected by RPL. The loss of the unborn child can be the most traumatic experience in a woman's life and is associated with significant psychological distress besides the instant grief. RPL can also be stressful for the partner, even though being at a lower risk for psychiatric morbidities. The man's gender role expects him to support and not to show weakness which may result in a suppression of his feelings and a disenfranchised grief.
Study design, size, duration: Cross-sectional study. All women and men referred to the special unit for RPL between March 2019 and October 2020 were asked to participate voluntarily with a total sample size of 105 couples and 17 women. Exclusion criteria were less than two pregnancy losses, inadequate knowledge of the German language and incomplete data.
Participants/materials, setting, methods: Couples were invited to fill out a questionnaire package estimating their psychological risks (e.g. posttraumatic stress disorder, anxiety, depression, perceived social support) and coping strategies with established instruments. Clinical history was obtained from medical records. Couple data were analysed with the Actor Partner Interdependence Model, taking the couple as the unit of analysis.
Main results and the role of chance: The response rate was 82.3%. The following psychological risks were measured among women versus men: posttraumatic stress disorder (PTSD): 13.7% versus 3.9% ($p=0.017$); anxiety: 50.4% versus 17.3% ($p<0.001$), depression: 48.1% versus 14.4% ($p<0.001$), lack of social support: 32.5% versus 32.7% (N.S.). A risk in at least one category showed 68.9% of women versus 44.8% of men ($p<0.001$), with those at higher risk indicating a lower satisfaction with their partnership ($p<0.001$) and higher impairment of their sexual life ($p<0.001$). Neither total number of pregnancy losses nor time gap since last pregnancy loss showed relevant correlations with psychological risks. For women, number of curettages, controlled for the number of pregnancy losses, correlates with the severity of posttraumatic stress ($p<0.05$). Higher levels of anxiety, depression and a lack of social support in women correlated positively with posttraumatic stress in their partners. The coping strategy "trivialization and wishful thinking" as well as the subscale "avoidance" of the Impact-of-Event-Scale (self-report questionnaire measuring posttraumatic stress) of men was correlated with more severe posttraumatic stress in their female partners (both $p<0.05$).
Limitations, reasons for caution: The data show only correlations between the measured variables, as cross-sectional studies are not suitable to analyse causal relationships. The sample was obtained in a special unit for RPL at a university hospital, so the findings may not be generalizable to all couples with RPL.
Wider implications of the findings: Screening psychological risks in couples with RPL may be reasonable considering the high risks in both sexes and the extent of PTSD diagnoses in women, their interdependencies and the potential risk of chronification. Professionals should encourage affected couples to support each other and provide targeted information on mental health services.
Trial registration number: DRKS 00014965

P-491 The mediator role of pain-related psychological inflexibility in the relationship between psychopathological symptoms and pain intensity in endometriosis

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Study question: Does pain-related psychological inflexibility play a role in the relationship between psychopathological symptoms (depression, anxiety, stress) and pain intensity in women with endometriosis?
Summary answer: Pain-related psychological inflexibility acts as a mediator exclusively between depressive symptoms and pain intensity. Psychopathological symptoms did not reveal a direct effect on pain intensity.
What is known already: Endometriosis is a chronic and incapacitating condition frequently involving the experience of pain (e.g., dysmenorrhea, ovulation pain, dyspareunia, chronic pelvic pain). Women dealing with endometriosis may present impaired health-related quality of life and psychological distress, with depressive, anxiety, and stress symptoms being commonly reported. Pain-related psychological inflexibility involves emotion regulation processes, such as avoidance of pain and cognitive fusion with pain. Cognitive and behavioural processes influence the relationship between pain and psychopathological symptoms, and pain-related psychological inflexibility showed to be an underlying mechanism in this relationship.
Study design, size, duration: Cross-sectional study. Participants' recruitment was completed through the Associação Portuguesa de Apoio a Mulheres com Endometriose and the Associação Portuguesa de Fertilidade (endometriosis and infertility patients' associations). Inclusion criteria were age (18 years or older) and an endometriosis medical diagnosis (self-reported). Data collection occurred between February 2018 and May 2018.
Participants/materials, setting, methods: A sample encompassing 209 women with an endometriosis diagnosis completed online a sociodemographic questionnaire, the Depression, Anxiety and Stress Scales (DASS – 21), the Numeric Pain Rating Scale (NPRS), and the Psychological Inflexibility in Pain Scale (PIPS-PT). Descriptive and correlational analyses were carried out using SPSS v. 26, and path analyses were estimated in AMOS (v. 24) with bootstrap procedures (2000 samples).
Main results and the role of chance: Participants' age ranged from 18 to 50 years old with a mean of 34.03 (SD = 6.44) years. The majority of participants were married (n = 112; 53.6 %), followed by single (n = 54; 25.8 %). Regarding years of education, a mean of 14.62 years (SD = 2.80) was found. Participants reported that their endometriosis diagnosis had been established for 4.55 years (SD = 4.56). Correlation analyses showed that depressive, anxiety and stress symptoms were significantly and positively associated with pain intensity and pain-related psychological inflexibility. A mediation analysis was conducted to examine whether pain-related psychological inflexibility mediated the effect of psychopathological symptoms on pain intensity. Paths showing not to be statistically significant were removed. The final model defining an effect of depressive symptoms on pain intensity mediated by pain-related psychological inflexibility explained 26% of the variance. This model showed a good fit to the empirical data: $\chi2(5) = 10.75$, $p = .057$, CMIN/DF = 2.15; TLI = .98; CFI = .99; RMSEA = .07, 95% CI = .00 to .14. Depressive symptoms predicted elevated pain intensity fully through higher pain-related psychological inflexibility (b = .05; SEb = .01; Z = 8.45; p < .001; β = .51).
Limitations, reasons for caution: Although path analysis is a powerful statistical technique, our findings rely on cross-sectional and self-report data. The study was disseminated through patients' associations, limiting the inclusion of people who do get in touch with such organizations. Moreover, online recruitment tends to recruit participants with more access to online platforms.
Wider implications of the findings: Pain-related psychological inflexibility seems to be a relevant construct to be addressed in the psychological assessment of women dealing with endometriosis. Furthermore, results suggest the

relevance of targeting emotion regulation processes, and not only focus on reducing pain, in pain management interventions.
Trial registration number: N/A.

P-492 Knowledge about reproductive health among cohort of oocyte donors in Spain

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Study question: What degree of reproductive health knowledge have oocyte donors?
Summary answer: The results of this study reveal that although oocyte donors are aware of the risks of possible fertility disorders, reproductive health knowledge is insufficient
What is known already: Sterility affects approximately 15% of the population of reproductive age, that is, young people. However, the information that young people have about fertility is scarce. Gamete donors are a group especially involved in reproductive issues since they help many people to solve their fertility problems and must undergo numerous tests before being accepted as such. However, there are no studies in Spain that deal with the knowledge that young people and, more specifically, donors, have about reproductive health and fertility
Study design, size, duration: A prospective, cross-sectional multicenter study including oocyte donors at ten fertility clinics performing gamete donation treatment in Spain. During a 2-month period (September-October 2020), 63 donors aged between 19 and 35 years old were recruited consecutively and a total of 63 oocyte donors were included as sample population. Most of them (78%) had not donated before
Participants/materials, setting, methods: 54% oocyte donors had secondary education and 43% have achieved university studies. Participants anonymously completed a questionnaire containing 41 questions divided into three sections: sociodemographic characteristics (11 items), knowledge on fertility and reproduction (22 items) and with a Likert scale, response to determine general reproductive health information as well as known risks for fertility disorders (8 items).

Besides descriptive statistics, statistical analysis was performed with Chi square test. $p < 0.05$ was considered significant
Main results and the role of chance: In the survey 96.8% of the participants reported that they had already known the tests for fertility disorders.

The increasing age of the women was correctly assessed by the participants of the study as a decisive risk factor for fertility, but it was found that exact knowledge was lacking: the decrease of a woman's fertility by 39.7% was stated to occur on average at the age of 35-40 and by 30% at 40-45. Nevertheless, 66% of donors considered that fertility preservation should be carried out before the age of 35.

61.1% of the non-university donors reported that fertility can drop as a woman ages due to the decreasing number and quality of the remaining eggs. Among university donors, this percentage increases to 92,6% (p:0,034). Merely 47 % of the participants informed what they understood that ovarian reserve is and 47.6% of donors believed that women create new eggs every month.

Regarding the known risk factors for fertility, lifestyle was mentioned most frequently by all participants (91,2%), followed by chemo/radiotherapy (83,8%) and smoking, alcohol, and drugs (82,4%). Concerning the influence of the body mass index on fertility, differences were found between non-university (61%) and university donors (88,9%) (p:0,012).

Limitations, reasons for caution: Financial compensation has been found to be a motivating factor for oocyte donors and therefore one could question the representativeness of the participating oocyte donors. It would be of great interest to explore the significance of the financial compensation further.
Wider implications of the findings: The present study reveals an existing requirement for information among oocyte donors, which is not only important for the success of prevention plans but also provides a foundation for possible strategies for the prevention of fertility disorder.
Trial registration number: not applicable

P-493 Couples joint narratives of coping with and making sense of recurrent pregnancy loss: a dyadic interview study

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Study question: What are couples' joint narratives of coping with, and making sense of recurrent pregnancy loss?
Summary answer: Couples can become stuck in patterns of communication and coping roles that may be dissatisfying and not reflect the complexity of their experience of RPL.
What is known already: Fertility problems such as recurrent pregnancy loss (RPL) are unique health issues because they are a couple problem, in that they involve a blocked parenthood goal for both members of the couple regardless of the cause or source of the fertility issue.

Previous research has focused on the psychosocial impact on the individual or examined gender differences in men and women's response to RPL. Research suggests that couples' relationships are impacted, but few examine this issue in interviews with couples as participants. We need appropriate study designs to examine and understand the couple's experience and process on a dyadic level.
Study design, size, duration: This was a qualitative study using dyadic interviews and analysis. This method facilitates a co-construction of meaning and joint narrative between couples through sharing and comparing their experience in a conjoint dyadic interview.

Thirteen couples who were referred to the RPL program, in Rigshospitalet, Copenhagen, Denmark were interviewed over a two-month period in 2017-2018. Interviews were held at Rigshospitalet and ranged between 81 and 109 minutes (average 91 minutes). Participants/materials, setting, methods: Inclusion criteria included: heterosexual couples with at least three pregnancy losses (PL) under 12 weeks gestation with no children/one child and willing to be interviewed in English. Thirty invitations were sent to couples who met the inclusion criteria and 15 couples contacted the interviewer to schedule an interview. Semi-structured dyadic interviews were conducted in person with 13 couples. Data was analyzed using dyadic analysis with a focus on common themes in co-constructed meaning across couples.
Main results and the role of chance: On average, participants had been in their relationship for 8.4 years, experienced three PLs (range three–six), with most recent PL occurring 4.3 months before the interview.

Couples described becoming stuck in patterns of communication and in rigid roles of coping and relating (e.g., the optimist, the emotional one) that could be dissatisfying and not meet their needs and not reflect the nuances and complexity of their experience of RPL. Common roles included the "optimist" versus the "pessimist", the "talker" versus the "listener" and the "emotional" versus the "rational / problem solver". While these roles were rooted in some truth of their experience, the rigidity of these roles did not create space and flexibility for the full spectrum of their reactions and experience. For example, a woman said, "*I hope for him to be the pessimist so I get to be the optimist sometimes*" and several men shared the depth of their grief for the first time.

The interviews were a way to highlight and create a new dynamic by allowing couples to respond to and correct their partner's assumptions about their experience or role (e.g., "that's not how I feel all the time") and try new ways of communicating.
Limitations, reasons for caution: The findings cannot be generalized to all couples who have experienced RPL given the study design. Whilst dyadic

interviews allow for a fuller, more nuanced narrative account, couples may omit some of their experience in the interview due to social desirability.

Wider implications of the findings: This study provides a better understanding of the complexity of communication patterns and roles in couples with RPL that can improve provision of support and counselling.

Dyadic interviews can provide opportunities for couples to communicate differently and break out of dissatisfying patterns while creating common ground and generating shared meaning.

Trial registration number: N/A

P-494 The relationship between the infertility specialist and the patient during the COVID-19 pandemic

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Study question: What are the basic elements of the infertility specialist - patient relationship?

Summary answer: For study participants, elements such as empathy, communication, collaboration and trust are important factors in increasing compliance and satisfaction with treatment.

What is known already: In the literature, the doctor-patient relationship is seen as fundamental in the treatment of infertility, due to the emotional implications of fertilization procedures. However, not much data is available specifically for this relationship. Communication and interpersonal skills, empathy, trust, collaboration, compliance and satisfaction are essential elements in this medical context.

Study design, size, duration: A cross-sectional study was conducted between May and June 2020 and follows the methods of a quantitative analysis, the data being collected using questionnaires. The research plan is one specific to the path analysis with mediation effect, in which the hypotheses were tested.

Participants/materials, setting, methods: The research group consists of 151 women diagnosed with infertility, voluntarily recruited through online support communities. Self-reported questionnaires provided socio-demographic information, information related to infertility, as well as information on the relationship of patients with specialists. In order to test the proposed hypotheses, an analysis of structural equations was performed, using the AMOS 20 program.

Main results and the role of chance: According to statistical indicators, CMIN / DF = 2.124, RMSEA = .087 and the p value of the Chi-square is less than 5% significance level, the model proposed is accepted. Based on the reported NFI and CFI values (.777 and .867), as well as the values of RFI = .763, IFI = .868 and TLI = .859, it can be concluded that the model is appropriate for the sample data. The p value of all constructors is less than .05, except for trust in doctor - cognitive empathy and patient satisfaction. All the others paths are significant: the effect of communication on treatment compliance, mediated by trust in specialist and collaboration; the effect on communication on treatment compliance, mediated by affective empathy, trust in specialist and collaboration; the effect on communication on patient satisfaction, mediated by affective empathy and trust in doctor; the direct effect of communication on trust in doctor, as well as the mediated effect by affective empathy. Regarding the relationship with doctor during the COVID-19 pandemic, 33% of the study participants stated that the pandemic affected the relationship with doctor, and 44% discontinued contact with specialist and medical procedures between March and June 2020.

Limitations, reasons for caution: Only infertile, voluntary women participated in this study, which limits the generalizability to other populations. Because the data are cross-sectional and correlational, the direction of causation is not proven. Additional experimental research on larger populations is needed to examine the effects of communication on patient satisfaction and compliance.

Wider implications of the findings: The study draws attention to the importance of basic concepts in the relationship of specialists with infertile patients. Thus, it is necessary for health care providers in assisted human reproduction to participate in programs for the continuous training of empathic communication skills, given the sensitivity of this diagnosis.

Trial registration number: Not applicable

P-495 Telemedicine in ART during SARS-CoV-2 pandemic : far and yet close

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Study question: Could telemedicine help in the management of the infertile couple's path at a fertility center?

Summary answer: The introduction of telemedicine increased the number of cycles within 6 months from the first consultation and reduced the drop-out rate.

What is known already: In Italy during the complete lockdown due to the first pandemic wave of SARS-CoV-2 the activity of fertility centers were stopped, with the exception of fertility preservation procedures for oncological patients. We therefore proposed a service of telemedicine to our patients, that we called SmartPMA.

Study design, size, duration: A longitudinal study performed at a public infertility center. The SmartPMA service was offered to 93 couples referred to our center from March 9th to May 31st, 2020. Initially 72 couples was interested in SmartPMA. Our center gradually re-opened and the first oocytes retrieval was performed on June 9th .

Participants/materials, setting, methods: Sixty-one out of 72 couples (85%) performed the SmartPMA from April 7th to June 16th, 2020. After acquiring informed consent and sending two anamnestic questionnaires, we booked a video-consultation with a gynecologist and a biologist. Afterwards, we sent medical prescriptions for appropriate clinical tests. At the re-opening, we offered the chance to start the ART cycle. Time to the first treatment and drop-out rates were compared to historical controls (2017-2019) using chi-square test.

Main results and the role of chance: Eleven couples declined the SmartPMA and booked an appointment at the reopening. Only 2 of these couples (18%) actually performed an IVF treatment within six months.

Three out the 61 couples that accepted the SmartPMA did not perform IVF treatments because the age of women exceeded the legal limit to access to a public ART center. Twelve out of 58 couples (21%) did not performed any IVF treatment for the following reasons: 4 women spontaneously got pregnant, 1 couple gave up for medical reasons, 3 were referred to ovodonation, and 4 were lost to follow-up.

Thirty-eight out of 58 couples (66%) (median age of woman: 36 + 4 years, range 27-43) performed at least one ART treatment (14 IUI, 12 ICSI cycle, 12 FIVET cycle). Eight out of 58 couples (14%) needed further clinical tests and their treatments are ongoing. The mean time to first treatment in the SmartPMA couples was 4 + 1 months (range 1-6). After SmartPMA, 66% of the couples performed the first cycle within 6 months, compared to 37% of controls (333/898 couples at their first access to our center from 2017 to 2019), p <0.00001. The drop-out was reduced from 39% to 20%, p=0 .0038.

Limitations, reasons for caution: We cannot exclude that the couples that joined the SmartPMA service during the pandemic period were particularly motivated to perform IVF treatments. We are aware of the small sample size and that this is a monocentric study.

Wider implications of the findings: Even after the pandemic, telemedicine can be an useful tool for fertility centers to reduce the discomfort of several visits in hospital, without losing patients but rather ultimately reducing the time to treatments and drop-out.

Trial registration number: not applicable

P-496 Alpha test results: Towards developing a digital prototype intervention to support parents' disclosure about donor conception in the United States

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Study question: The objective of the study was to determine the usability, comprehensibility, and acceptability of a digital, decision-support aid prototype supporting parental disclosure of donor conception. Summary answer: This mixed methods design maximized participant feedback about the digital, decision-support aid prototype yielding rich insight about the prototype while minimizing participant and investigator burden.
What is known already: Although a paradigm shift is underway to remove the secrecy that has historically shrouded the practice of gamete donation, little is known about best practices that can support parents in disclosing the conceptional origins to their children. To address this gap, we created a decision-support aid prototype to facilitate parental disclosure post-treatment. In doing so, we followed the International Patient Decision Aid Standards Collaboration for developing decision-support aids, which recommends that Alpha testing (i.e., usability, comprehensibility, and acceptability) be completed to improve the quality of newly created decision-support interventions prior to Beta (i.e., real world) testing.
Study design, size, duration: A mixed-methods, triangulation design was used.
Participants/materials, setting, methods: Sixteen participants were purposefully selected based on desired characteristics and the needs of the study. Participants were asked to: (1) follow investigator prepared instructions for accessing the digital, decision-support aid; (2) complete a quantitative rating form about each slide within the decision-support aid prototype; and (3) participate in a qualitative, cognitive interview or focus group. Descriptive statistics and qualitative content analysis guided the iterative analysis.
Main results and the role of chance: The sample was composed of 10 parents that used donated sperm (n = 3), oocytes (n = 4), or embryos (n = 2) to conceive children and 8 clinicians; 2 participants were both donor recipient parents and clinicians. The interviews (n = 14) and one focus group (2 participants) ranged in length from 25 to 70 minutes (M = 47 minutes).
Usability: Participants suggested refining the instructions for accessing the decision-support aid and upgrading the technology used to deliver the content. Common concerns were the inconsistent volume of the audio recordings and a need for higher quality images. Comprehensibility: Feedback obtained from the participants' rating forms and in the interviews and focus group were consistently high about the ability to understand the content and the scope of the information presented. Acceptability: Participants noted the aid would resonate with parents. They recommended shortening the length of the aid, changing specific wording, modifying some of the video content, refining specific content for individual slides and the four modules. Unintended recommendations about how the aid might be used to provide clinician education or in international research were also reported.
Limitations, reasons for caution: Alpha testing is not designed to obtain all possible technological or content issues. Rather, it is a useful and recommended step in intervention development to mitigate existing technological bugs and key content issues prior to implementation of Beta testing of a decision-support aid.
Wider implications of the findings: Other investigators that develop digital decision-support aids may consider the use of both quantitative and qualitative data collection methods during Alpha testing to refine digital interventions efficiently. The use of mixed methods not only captures rich and insightful feedback but also minimizes the burden on participants and investigators.
Trial registration number: Not Applicable

P-497 Anxiety, depression and sexual dysfunction among women with genital diseases

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Study question: How is the prevalence of anxiety, depression and sexual dysfunction among Chinese women who is suffering from genital diseases? What is the relationship among their anxiety, depression and sexual dysfunction?
Summary answer: Anxiety, depression and sexual dysfunction symptoms were self-reported by the participants. Besides, their anxiety, depression and sexual dysfunction were inter-correlated.
What is known already: Reduced sexual activity and dysfunctional problems are highly prevalent in females. Approximately 43% of American women reported experiencing sexual problems.

Women who are facing pressure on childbirth, may be more likely to have mental health problems. Some studies have shown that having gynecological disease can lead to anxiety, depression and sexual dysfunction. However, the mental health of females who are suffering from genital diseases has been little studied.
Study design, size, duration: The investigation was carried out from March to November, 2020. Participants were recruited in the Hong Kong University-Shenzhen Hospital, located in Shenzhen, China. 135 patients were approached while 116 agreed to join the survey.
Participants/materials, setting, methods: 116 women (35.42±8.19 years old) with a diagnosis of fallopian tube disease, ovarian benign disease or uterine disease voluntarily filled a questionnaire which contains the Female Sexual Function Index-6 Items (FSFI-6) and the Hospital Anxiety and Depression Scale (HADS). Descriptive analysis and stepwise regression were used to present participants' self-reported anxiety, depression, and sexual dysfunction problems, as well as the relationship among their anxiety, depression, and sexual dysfunction.
Main results and the role of chance: 25% of the participants reported anxiety symptoms; 9.5% of the women reported depressive symptoms; while 37.3% reported sexual dysfunction problems. Anxiety ($p<0.001$) was significantly associated with depression. Depressive symptom can significantly predict sexual dysfunction ($p<0.05$) while anxiety was not associated with sexual dysfunction ($p>0.05$). Targeted interventions are needed to improve the mental health status of women with genital diseases in China.
Limitations, reasons for caution: This study can only present mental health status of this population. To better show the odds ratio of mental health problems, a case-control study design is needed. Besides, future qualitative or longitudinal studies are needed to detect the risk factors for the poor mental health of women with genital diseases.
Wider implications of the findings: Reduced sexual activity and dysfunctional problems are highly prevalent in females with genital diseases. Sexuality is an important element in patients' quality of life. For female patients with genital diseases, we should not only treat their physical symptoms, but also guide and treat patients in mental ways.
Trial registration number: ChiCTR2000031343

P-498 Patient's pregnancy rates after IVF fresh embryo transfer positively correlates to the number of visual connections to live blastocyst development images of own embryos

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Study question: Looking at time-lapse images of own embryos during the five days of culture, as a patient's active behaviour, may help to increase pregnancy rates?
Summary answer: The number of visual connections to live blastocyst development images of own embryos positively correlates to patient's pregnancy rates after fresh single blastocyst transfer.
What is known already: In human reproduction, despite the evidence that infertility itself and reproductive treatments can produce anxiety and stress, the impact of emotions on pregnancy outcomes is unknown and probably underestimated. The interaction between psyche, nervous, immune and endocrine systems (PNIE) is well known and has been applied to understand and treat different pathological conditions. Patient's active behaviour during IVF treatments appears to be a positive strategy to decrease women's anxiety and stress, thus to increase fertility quality of life and pregnancy outcomes.
Study design, size, duration: Retrospective comparative study of 934 patients undergoing fresh IVF cycles during three years. To avoid any bias related

to oocyte and embryo factors, we only included egg donation cycles in the study, and ≥3BB (Gardner score) single embryo transfers. All embryo cultures were performed in time-lapse incubator and patients could connect on-line to their embryo's images before single blastocyst transfer. We evaluated the impact of visual connections to live embryo images on pregnancy rates.

Participants/materials, setting, methods: On day one of embryo culture, consenting patients received an individualized and secure link allowing them to connect on-line to images of their own embryos any time during the five days of culture. Patients were divided in five groups depending on the number of on-line visualisations (A=0; B=1-10; C=11-20; D=21-30; E>30). Pregnancy and clinical pregnancy rates were compared between the five groups. Chi-square test for a large contingency table was performed to compare all groups

Main results and the role of chance: The distribution of patients in the five groups, based on number of visualizations, resulted as follows: 287 in group A; 328 in group B; 156 in group C; 80 in group D; 83 in group E.

The five groups were homogeneous and there were no statistically significant differences in recipient's ages (A 42.8±3.9 years; B 41.9±3.9 years; C 41.8±4.5 years; D 42.8±4.1 years; E 41.9±4.4 years) and in donor's ages (A 25.5±4.3 years; B 26.9±4.4 years; C 27.1±4.2 years; D 26.4±4.5 years; E 26.5±4.1 years) among the five groups.

We observed a progressive positive trend between the number of on-line visualisations and pregnancy rates, reaching statistical significance for group E (>30 visualizations) compared to the others groups. In group E, pregnancy rates and clinical pregnancy rates per fresh single blastocyst transfer were respectively 72.3% and 65.1%, significantly higher when compared to the others groups ($p<0.01$, $p<0.001$): group A 61% and 50.9%, group B 63.1% and 56.1%, group C 64.1% and 55.1%, group D 65% and 53.8%.

Limitations, reasons for caution: Time-lapse incubator is needed as well as a strong informatics setting to ensure secure and personalised on-line connection to each patient at any time during the five days of culture. Endocrine and endometrial biomarkers should be proposed and evaluated in further clinical trials to better understand these results.

Wider implications of the findings: Repeated on-line visualization of own embryos images before fresh blastocyst transfer enhances pregnancy rates in IVF cycles. We propose that repeated visual stimuli of images produces a positive emotional connection between patients and their own developing embryos, thus reducing anxiety and enhancing recipient's endometrial receptivity.

Trial registration number: Not applicable

P-499 infertility impact on perceived quality of life and sexual satisfaction in Spanish women with primary and secondary infertility

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Study question: Are there any differences relating to the perceived quality of life (QoL) and sexual satisfaction among infertile women?

Summary answer: There were no differences between women who already had a baby and those who did not relating to the perceived QoL and sexual satisfaction.

What is known already: Infertility is a medical disease with a high social component with a 16% prevalence. There have been many investigations regarding to the physical part of the infertility but the sexual and marital satisfaction has not been as intensively investigated. The importance of the psychological counseling in fertility treatments has already been proven, but the significance of sexual satisfaction on individual's perception on QoL has not been as deeply studied.

Study design, size, duration: A transversal descriptive study was done. 313 heterosexual married women with fertility problems were recruited in collaboration with the Spanish patient association "*Red Nacional de Infértiles*". The Fertility quality of life tool (FertiQoL) was selected to measure the perceived QoL and the Index of Sexual Satisfaction (ISS) was chosen to study the degree of sexual satisfaction. The data collection was made between January and February 2020 and all the information was gathered online.

Participants/materials, setting, methods: 313 women filled the questionnaire which had 4 different modules: A sociodemographic module (sex, age, studies, time trying to conceive, moment of treatment and offspring), two modules for each measurement instrument and a last module in which they could write their personal experiences regarding to the infertility journey. ANOVA and t-Student statistical analyses were done to compare the different independent variables. To see if FertiQoL could explain the sexual satisfaction a regression analysis was made.

Main results and the role of chance: To achieve 95% power ($\alpha=0.05$) and an effect size of 0.25, a minimum sample size of 210 was needed and a sample of 313 women was recruited. There were no statistical differences between women with previous offspring and those who did not in neither of the FertiQoL subscales (Emotional: 7,4 ± 3,884 vs. 7,34 ± 4,235; Mind/Body: 9,65 ± 5,098 vs. 8,66 ± 4,979; Relational: 16,88 ± 4,807 vs. 16,3 ± 4,956; Social: 10,52 ± 5,02 vs. 10,1 ± 4,801; Tolerability: 5,91 ± 4,114 vs. 6,65 ± 3,357; Environment: 12,71 ± 5,02 vs. 11,42 ± 4,963) nor in the ISS questionnaire (47,48 ± 6,488 vs. 47,22 ± 7,35). Regarding to the power of the FertiQoL instrument and the perceived QoL to predict the sexual satisfaction, the regression model showed that the sexual satisfaction could be explained in 26,3% of the cases by the relational and mind/body subscales of the FertiQol tool. This model showed the inherent relationship between marital and personal wellbeing in order to obtain a better sexual satisfaction.

Limitations, reasons for caution: As the study had a transversal design, no cause-effect relationships could be done. It would be desirable to establish a longitudinal study in order to determine a more accurate relationship between the studied variables.

Wider implications of the findings: This study showed that the impact of infertility in women with secondary infertility diagnose could be at least as high as in women with primary infertility diagnose. FertiQoL would be a reasonable instrument to estimate the sexual satisfaction of infertile women. Sexology should be part of the infertility counselling programs.

Trial registration number: Not applicable

P-500 Euploid embryo-transfer reduces advanced maternal age patients' anxiety in the waiting period before the pregnancy-test.

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Study question: Can PGT-A reduce the anxiety generally experienced by infertile women undergoing IVF in the waiting period between embryo transfer and the pregnancy test?

Summary answer: PGT-A reduces anxiety in infertile women after embryo transfer, probably due to a gain of confidence in their treatment route.

What is known already: The waiting period, i.e. the time between embryo-transfer and the pregnancy-test, is considered unpredictable and unmanageable, thus figuring amongst the most stressful steps of an IVF treatment. This is mainly imputable to women's lost sense of control over the outcome. Uncertainty is in fact a source of fear and elevated distress. PGT-A has been shown to improve live birth rate per embryo transfer and reduce miscarriage rate per clinical pregnancy across several trials and observational studies worldwide, especially in advanced maternal age (AMA) women. Here, we investigated if euploid embryo transfer does involve also lower emotional burden over untested one.

Study design, size, duration: Prospective observational study evaluating the level of anxiety in the waiting period among women undergoing euploid or untested embryo transfer. Data were collected between September 2019 and September 2020 in a public hospital. A total of 48 infertile women were recruited: 25 undergoing euploid single embryo transfer after trophectoderm biopsy and NGS, and 23 undergoing untested single embryo transfer.

Participants/materials, setting, methods: To measure the level of anxiety, the two groups completed the STAI (State Trait Anxiety Inventory) questionnaire

at two time points: before starting the ovarian stimulation (T0), and at day 8 after embryo transfer (T1). The chosen questionnaire has been previously validated to capture the level of patients' anxiety during the waiting period. Outcomes of T0 were used to control for individual level state of anxiety at T1.
Main results and the role of chance: The two groups showed similar reproductive history and sociodemographic characteristics except for female age, which was higher in the PGT-A group (37.7±3.2 yr versus 32.3±2.2 yr in the control). This is due to AMA (maternal age >35 yr) being the main indication to PGT-A. Conversely, the duration of infertility was similar in the two groups (3.8±2.2 yr versus 3.7±1.9 in the control). At T0 all patients showed similar levels of anxiety (46.4 points versus 49.9 in the control, 95%CI of the difference: from -9.97 to 3.03 points, p=0.3). Remarkably, at T1 instead, the women undergoing euploid embryo transfer showed a significantly decreased level of anxiety with respect to the control (39.9 points versus 53.4; 95% CI of the difference: from -18.26 to -8.69, p<0.01). This difference remained significant also after controlling for the baseline value at T0, and adjusting for potential confounding factors in a multivariate analysis (adjusted p-value<0.01).
Limitations, reasons for caution: The sample size is small, yet the study resulted powered enough to reveal the considerable advantage of PGT-A toward the primary outcome. We analysed only the waiting period here. Therefore, data will be collected in the future at subsequent gestational stages, such as when prenatal genetic diagnosis is usually conducted.
Wider implications of the findings: Women undergoing PGT-A seem reassured by the technique. This is probably due to the gain of confidence and control derived from an increased expectation of success. From this perspective, assessing women's wellbeing and attitude towards all different clinical procedures should become a critical part of their treatment.
Trial registration number: None

P-501 Deep in the Maze: The psychosocial trajectory and decision making of Women with recurrent implantation failure of IVF

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Study question: To understand the psychosocial trajectory of Chinese women who have experienced recurrent implantation failure (RIF) of IVF and their decision making accordingly.
Summary answer: Chinese women experience despair, doubt, and disorientation along with the cumulative failure cycles of IVF, and stick to IVF as the ultimate option.
What is known already: Recurrent implantation failure, the absence of implantation after repeated embryo transfers is a stressful event for people undergoing treatment for infertility. Numerous researches have focused on the psychological wellness of women undertaking IVF, but pay less attention to the subgroup who have undergone repeated failures. Current studies have shown that women after repeated unsuccessful IVF might endure anxiety, depression, and other psychosocial distress; however, the feelings brought by the different times of failure are unlike, and these experiences will affect their treatment decisions accordingly, which is rarely studied.
Study design, size, duration: Semi-structured interview was adopted with sixteen Chinese women from March 2020 to July 2020. The interview lasted 90 minutes. A follow-up survey was conducted three months after the interview. Driven by grounded theory, data is analyzed by thematic analysis.
Participants/materials, setting, methods: Convenience sampling was used to recruit female participants who have failed to achieve clinical pregnancy after two consecutive cycles of fresh or frozen IVF embryo transfers with a cumulative number of transferred embryos of four or more cleavage-stage embryos or two or more blastocysts. Chinese-speaking women who were undertaking IVF treatment in the reproductive center of hospital in Shenzhen City were recruited by pamphlets and doctors' referral.
Main results and the role of chance: Chinese women with recurrent implantation failures experience the following psychosocial trajectory during the cumulative failure cycles of IVF: despair, doubt, and disorientation. Despair usually comes after the first failure: the high expectation for success rates makes the initial failure exceptionally shocking and desperate. Huge distress brings two kinds of decisions: start a new IVF cycle quickly to welcome the positive results in the imagination, or wait for a period of time to avoid the pain of failure again. Doubt usually appears after the second failure. In addition to doubting the health function of their body, recurrent failure makes the patients particularly doubt the efficacy of IVF and doctors' clinical judgment. Some patients would do 'hospital shopping' and consider change clinics. Disorientation can be seen in patients who have experienced more than three cycles of failure. Past experience and meaning cannot help them understand and solve the current dilemma. The inherent concept of fertility continues to strengthen their belief of having a baby as ultimate goal. The follow-up survey found that most women still choose to continue IVF treatment after repeated failure. They are like being in the maze of fertility, wandering for a long time but unable to get out.
Limitations, reasons for caution: Several limitations are identified: self-selection bias due to convenience sampling; narrow sampling approach may limits the generalizability; the exclusion of men may ignore the marital interdependence during the infertility treatment.
Wider implications of the findings: Patients who have experienced recurrent implantation failure demand promising intervention during and after infertility treatment. The findings demonstrate the need for both supportive and implication counseling to facilitate them cope with the psychosocial distress, and make value-based decision making, so as to enhance their self-agency.
Trial registration number: not applicable

P-502 Reproductive specialists should be offered additional training in providing emotional support to patients

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Study question: Should medical professionals working in fertility care have additional training on how to deliver bad news and provide emotional support to patients?
Summary answer: Medical professionals providing assisted reproduction treatments should be offered more training to better assist the emotional burden that accompanies the treatment as described by patients.
What is known already: Pursuing fertility treatment provides a large physical and medical burden on patients. Many patients describe going through IVF like having a second full time job, adding stress, anxiety and social pressure to an already difficult time of trying to conceive. As specialists in reproductive treatment the main focus is trying to assist patients in achieving a healthy pregnancy and baby. In recent years it's become evident that the emotional aspect is equally important and often patients are left feeling that their emotions and concerns are dismissed by the very healthcare professionals that are trying to help them have a baby.
Study design, size, duration: This qualitative study recruited 100 women who have had received assisted reproductive treatment in the United Kingdom during 2020. An online survey was presented to women who fit the inclusion criteria and and recruitment ended when data saturation was reached. The survey design consisted of open-text qualitative answer format.
Participants/materials, setting, methods: Inclusion criteria were women aged between 24-45 years old who had an infertility diagnosis and received assisted reproductive treatment. A survey was presented to them to describe any negative communication they had from medical professionals, specifically what was said and how it made them feel.
Main results and the role of chance: The most frequent answers were "you're still young" (26%), "just lose some weight" (18%) and "it only takes one" (10%). Additional information included in responses had a message of "don't worry" (50%) and "just relax" (32%). 20 participants had experienced miscarriage and 8 of them had been told "at least you can get pregnant". The emotional response that accompanied messages like these from their healthcare professionals included anger, frustration and discouragement from "feeling invalidated", "my feelings don't matter" and "feeling like my baby didn't matter".
Limitations, reasons for caution: As the results are based on qualitative data from 100 women, the results can not be readily generated to larger populations. Furthermore, the questionnaire was specifically focused on negative experiences and therefore bias would have occurred. The survey was only advertised on social media.
Wider implications of the findings: Women undergoing fertility treatment commonly experience guilt, shame and lack of confidence. Given the question

was open ended and similar responses were given, indicates that additional training in emotional support throughout treatment would be recommended and stresses the need to better integrate these aspects of patient care into daily practice.

Trial registration number: Not applicable

P-503 Focus groups with health care professionals, patient advocates and patients to explore how the potential need for multiple cycles is managed during fertility treatment consultations

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Study question: How is possibility of failure and potential need for multiple cycles discussed with patients during the first or repeat IVF/ICSI treatment consultation?

Summary answer: Health Care Professionals plan treatment on a cycle-by-cycle basis because it is the normative way to plan treatment, but patients see advantages in multi-cycle planning.

What is known already: Many patients need more than one round of IVF/ICSI stimulation to achieve their parenthood goals. A recent study has found around 60% of patients to be willing to plan for multiple cycles of treatment. However, it is not clear how patients are informed fully about the high possibility of treatment failure and the subsequent need for multiple cycles during their treatment planning consultations.

Study design, size, duration: Qualitative focus groups with health care professionals (HCP) patient advocates (April 2020) and patients (July and August 2020, respectively). Patients were eligible if they had had a consultation to start a first/repeat stimulated IVF/ICSI cycle in the eight weeks prior to participation, were aged 18 or older (upper age limit of 42 years for women) and fluent in English. Eligible Health HCPs and patient advocates were those employed at a fertility clinic or charity, respectively.

Participants/materials, setting, methods: HCP, patient advocate and patient focus group topic guides started with general questions about fertility consultations and progressed to discuss if and how the possibility of treatment failure and need for multiple cycles was introduced and discussed, and then preferences regarding planning IVF/ICSI on a multi-cycle rather than a single cycle basis. Focus groups were recorded, transcribed and analysed using framework analysis which allowed examination of shared, unique and incongruent thematic content across participant groups.

Main results and the role of chance: Twelve HCPs, 2 patient advocates and 11 patients participated in seven semi-structured online focus group discussions. Framework analysis revealed 52 codes (e.g., possibility of failure tentatively introduced; discussion of multiple cycles dependent on clinical/patient benchmarks) abstracted into 17 higher-level categories (e.g., Failure is a sensitive topic to approach; IVF treatment failure is the norm). Synthesis of categories revealed four themes and one meta theme The meta theme showed planning treatment on a cycle-by-cycle basis was the norm. This meta-theme was supported by four themes: (1) 'culture of communication' that dictated benchmarks (e.g., clinic, national live birth rate) and definition of key concepts ('complete' cycle) that underpinned divergence between clinics; (2) 'HCP-patient dynamics' indexing degree of shared decision-making, advance preparation and involvement of partners in planning; (3) 'tempering optimism' that described tailoring, balancing and emotion management in giving personal chances of success; and (4) 'transitioning to multi-cycle planning' which identified worries of multi-cycle planning (e.g., need to learn from failure).

Limitations, reasons for caution: The majority of patients were women from private fertility clinics with no previous treatment experience recruited from social media websites, mainly associated with patient support groups. Similarly, the majority of HCPs were women from private fertility clinics. Informative comparisons across treatment stage, gender and funding source were therefore not possible.

Wider implications of the findings: HCPs are hesitant towards multi-cycle planning. However, patients show openness, suggesting a cultural shift from the single cycle norm of planning IVF/ICSI may be possible. If adopted by clinics, HCPs patients and fertility organisations, multi-cycle planning could encourage patients to create informed treatment expectations and plans prior to treatment engagemen.

Trial registration number: MS200059_001

P-504 A randomised controlled trial comparing expectant management or intrauterine-insemination in couples with unexplained subfertility and a poor prognosis for natural conception: the impact on health-related-quality-of-life

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Study question: Is health-related quality of life (HRQoL) in women with unexplained subfertility and a poor prognosis influenced by expectant management or intrauterine insemination with ovarian stimulation?

Summary answer: HRQoL did not differ, except for the relational domain which was lower after expectant management. Anxiety and depression disorders occurred frequently in both groups.

What is known already: In couples with unexplained subfertility and a poor prognosis, IUI with ovarian stimulation (IUI-OS) is a first line treatment. Not much is known about quality of live or depression and anxiety in these couples. The Fertility Quality of Life (FertiQoL) is reliable for assessment within relational and social domains, the Hospital Anxiety and Depression Scale (HADS) is a reliable tool to detect anxiety and depression disorders.

Study design, size, duration: We performed a multicentre RCT in couples with unexplained subfertility with a poor prognosis of conceiving naturally within one year. Women were allocated 1:1 to six months expectant management or to six months IUI-OS. HRQoL was assessed with standard self-administered psychometric measures with established reliability and validity: FertiQol and HADS. We intended to include 1091 couples but after almost 4 years, the study had to stop due to slow inclusion and therefore lack of funding.

Participants/materials, setting, methods: Between June 2017 and September 2020, we recruited 178 women of wich 92 were assigned expectant management and 86 IUI-OS. All women who participated and could read Dutch were eligible for the HRQoL measurements because HRQoL questionnaires in foreign languages were not yet available online. Women completed the questionnaires before randomisation, 3 and 6 months after randomisation. We used mixed model analyses to assess differences between treatment groups and the effect of time.

Main results and the role of chance: One hundred sixty-two women could read Dutch and were invited (162/178 (91%)). Analyzable data of the FertiQol questionnaire were available for 80% (130/162). Compared to women allocated to IUI-OS, women allocated to expectant management had a lower FertiQol score in the relational domain (mean difference -4.3 (95% CI -7.3 to -1.3) but not in the social domain (mean diff van -0.8 (95% CI -4.5 to 2.9).

Data of the HADS questionnaire were available of 156 women (96% (156/162)). Both groups had comparable scores in the Anxiety (mean difference -0.20; 95% CI 0.63; -0.99 to 0.6) and Depressions score (mean difference 0.002; 95% CI -0.67 to 0.67) at all three moments. At baseline, the incidence of an anxiety disorder (definition score 8 or higher) was 19% (30/156) and increased to 30% and 29% at 3 months and 6 months respectively. The incidence of a depression disorder (definition score 8 or higher) was 5% (7/156) and increased to 16% and 18% at 3 months and 6 months respectively. The incidences of anxiety or depression disorders did not differ significantly between expectant management and IUI.

Limitations, reasons for caution: Our randomized controlled trial did not reach the planned sample size. The results are only applicable to women with unexplained subfertility and a poor prognosis and not to all women with unexplained subfertility.

Wider implications of the findings: Although often assumed, IUI-OS does not improve HRQoL compared to expectant management in all domains. IUI might prevent loss of quality of the relationship, but the impact seems small. Future studies should look into the high incidence of anxiety and depression disorders in these women and how to support them.
Trial registration number: Trial register NL5455 (NTR5599)

P-505 The right age to tell? The insufficiency of the age criteria for characterizing the experience of French donor conceived families in disclosing to their offspring

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Study question: Is age a key criteria for characterizing the experience of families in telling donor offspring about the facts of their conception?
Summary answer: The study shows that, although donor offspring's age at the time of disclosure has an impact, it is insufficient to describe these families' experiences
What is known already: Secrecy was the norm for decades in donor conception, but "openness" has now become the new core value for institutions, professionals and interest groups. Accordingly, in recent years information-sharing practices have shifted in donor conceived families, but a proportion of parents, especially heterosexual couples, still appear to not inform their children about their being donor conceived. Disclosure recommendations seem difficult to apply in practice. A recurring question is: when should children be told? Age is presented as a key criteria: the younger the children are when their conception story is shared, the less of a problem it would create.
Study design, size, duration: The qualitative social science study includes two sets of semi-directive interviews conducted with 20 French sperm donor conceived adults (April-Dec. 2019) and 22 French parents by sperm, egg or double donation (Feb.-Oct. 2020). Calls for interviews aimed at donor conceived adults and parents by donation were shared on the Internet, in the media (press, radio, television) and through interest groups (PMAnonyme, BAMP!, MAIA) in France. The contact initiative was left to potential participants.
Participants/materials, setting, methods: Donor conceived participants include 17 women and 3 men conceived 1960-2000 through anonymous sperm donation in heteroparental families.

The parent participants include 20 families (20 mothers, 2 fathers) who used donor conception—mainly anonymous (19)—in France, Spain and the Czech Republic starting in the 1980s. 17 conceived as heteroparental couples, 2 as solo-mothers-by-choice and 1 as a same-sex couple. 17 have already informed their offspring of the facts of their conception.
Main results and the role of chance: The participants' experiences of disclosure appear to be bound to their historical and social context, especially regarding the prevailing norms on secrecy. Older parents mention having been advised by clinic professionals to keep the facts of their conception from their child(ren). Some also feared the stigma related to infertility. In contrast, some younger donor conceived participants recall the use of a children's book while being told of their conception as toddlers. Beyond age, the larger context thus affects information-sharing practices.

Furthermore, experiences of disclosure are impacted by the family context and history. Some are embedded within larger events such as divorces or the death of a family member. The story of the donation may be linked to narratives of diseases (such as cancer) or traumatic events (such as the loss of a fetus in utero) that may prevail over donor conception or make it untellable.

Age proves to be an insufficient criteria to qualify these experiences, all the more so since "disclosure" sometimes unfolds in several steps. Some parents have first talked about their fertility issues without mentioning the use of a donor. Behind the prevailing norm of "openness", difficulties in actually disclosing are confirmed.
Limitations, reasons for caution: Being qualitative, the study only includes a small number of participants without claiming exhaustivity nor representativity. It imperfectly reports on the view of those who do not disclose, as all participants question the principle of secrecy, many being members of interest groups defending openness.
Wider implications of the findings: Our results complement existing studies that emphasize the weight of age in donor conceived families' experience regarding disclosure. Age alone cannot describe information-sharing practices that are embedded within their historical and social context as well as the family context and history. Results thus inform familial difficulties related to disclosure.
Trial registration number: not applicable

P-506 Low anxiety and depression levels and a confronting attitude characterise infertile couples undergoing ART treatments during phases 2 and 3 of COVID-19 pandemic in Italy

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Study question: Which pshycological factors have influenced the choice of infertile couples seeking for ART treatment during Phases 2 and 3 of COVID-19 pandemic in Italy?
Summary answer: Couples undergoing ART treatment during Phases 2 and 3 of COVID-19 pandemic in Italy show low levels of anxiety and depression and a problem-centred attitude.
What is known already: COVID-19 pandemic has exposed people to psychological and health safety risks, forcing acceptance of important work and social restrictions. In lockdown Phase 1, all ART procedures were suddenly suspended. This was superimposed to the already critical emotional burden carried by infertile couples undergoing ART treatments. In our analysis performed in Phase 1, couples showed a logical consciousness regarding the need of such measures, given the evident risks, but the emotional drive was towards the continuity of the ART procedure. The emotional impact linked to the obligation of ART treatment suspension was in fact superior than that due to the lockdown.
Study design, size, duration: From July 7th to November 28th of 2020, an online form was made available to the patients of the Biogenesi Reproductive Medicine Centre of Monza and of the Eugin Fertility Clinics in Italy (Milano, Modena and Taranto), which was filled anonymously and voluntarily by 326 subjects.
Participants/materials, setting, methods: The questionnaire was composed by sociodemographic data, a Zung's scale for anxiety self-evaluation, a Zung's scale for depression self-evaluation, the COPE-NVI-25 questionnaire, five specific questions relative to what contributed for the decision of undergoing ART treatment during Phases 2 and 3 of the pandemic, and four specific questions relative to which support measures could have been efficacious to reduce stress. Main results and the role of chance: Of the 326 subjects included in the study, 19.9% were men and 80.1% were women; 77.3% underwent homologous and 22.7% underwent heterologous treatments; 44.5% lived in Lombardy, 20.9% in Liguria and the remaining 34.6% in different parts of Italy; 75.6% were seeking for maternity/paternity for less than 5 years and 24.4% for more than 5 years. The overall level of anxiety was low, as well as the level of depression, without any particular variations for gender or treatment type. The COPE-NVI-25 questionnaire revealed that couple strategies linked to a positive attitude towards the problem prevailed. What mostly contributed to ART treatment start or continuity in Phases 2 and 3 was the support to the couple agreement, couple age and the non-renounceable desire of having a child. Among the possible supporting measures aiming to reduce ART-related stress in this context we identified: greater support from the partner and inclusion of supporting treatments such as nutritional advises, massage and specifically driven physical activity.
Limitations, reasons for caution: The range of the present study is limited by the number of voluntaries and possible unprecise representation of the real ART patient population in Italy. The results presented could have been affected by variables that are uncontrolled for.
Wider implications of the findings: The findings contribute to understand the psychological status of infertile couples that start or resume ART treatments

under stressful conditions determined by the COVID-19 pandemic. These data shall provide valuable references for multidisciplinary discussion and strategy formulation aiming to improve the overall quality of ART treatments.
Trial registration number: not applicable

P-507 In It together: A Dyadic approach to assessing the health-related Quality of Life and Depression among infertile couples

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Study question: Is there a difference in perceived quality of life (QOL) and prevalence of depression between partners with infertility and to determine whether socio-demographic factors influence the same?

Summary answer: Among infertile couples, there was high degree of congruence in perceived quality of life and prevalence of depression was similar among partners

What is known already: Impairment of Quality of life and psychological ramifications of infertility are often not easy to recognize and are frequently overlooked by couples and clinicians alike. The focus of available studies is largely women's reaction to infertility and couple-based studies are limited at best especially in developing countries. Impact of socio-demographic factors on QOL and depression prevalence have not been studied in depth in couple-based studies.

Study design, size, duration: A prospective, cross-sectional study of infertile couples in setting of western India over a period of one year. In total, 130 couples (260 participants) attending the fertility clinic at a tertiary level teaching hospital were interviewed cross-sectionally, following due approval from the institutional ethical committee.
Participants/materials, setting, methods: Couples' socio-demographic and clinical details were recorded. Couples were requested to complete the WHOQOL-BREF instrument and Beck Depression Inventory (BDI). Analysis was performed using the statistical package SPSS, version 21, (International Business Machines Corp., Released 2012, Version 21.0.) and p-value of <0.05 was considered statistically significant. Data was analyzed using paired t-test, one-way multivariate linear variance analysis and regression and correlation models.
Main results and the role of chance: Out of 130 couples (260 participants), data 214 participants (107 couples) was included in Quality of Life score assessment and from 228 participants (114 couples) were included in the final depression analysis. Mean Quality of life (QOL) scores between men and women showed a strong agreement within psychological, social, environmental domains (r=0.70, 0.67 and 0.69 likewise) and moderate association for physical domain (r=0.59). Presence of depressive symptoms was associated with significantly impaired QOL scores through all domains. Depression was present in 30.6% of female partners with 18.4% having mild, 9.6% having moderate and 2.6% having severe depression. Corresponding figures in male partners were 27.2%, 20.2%, 6.1% with 0.9% case of severe depression. Pearson correlation between female partner BDI scores and male partner scores was highly statistically significant with a correlation coefficient of 0.745, significant at 0.01 level (99% confidence interval). Presence of depression was not found to be significantly associated with couples' age, education status, income status, presence of previous living child in the family and the duration of infertility using multinomial logistic regression model.
Limitations, reasons for caution: Being a questionnaire based study, there is predisposition to certain degree of inaccuracy of responses. The Cross-sectional design of the study allows estimation of variance and association but not causation.
Wider implications of the findings: Screening and psychoeducation should be couple based considering the couple as one unit which is likely to improve the mental wellbeing of the couple as a whole. All infertile couples should be screened and offered counselling irrespective of their socio-demographic background.
Trial registration number: AIIMS/IEC/2018/677

P-508 The relational self in fertility decision-making: Chinese lesbians exploring donor conception and biological ties

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Study question: How does the cultural importance attached to biological family ties shape Chinese lesbians' decision-making processes regarding whether and how to have children?
Summary answer: The cultural significance of biological ties shapes Chinese lesbians' fertility decisions, including those regarding conception methods, who will get pregnant, and whose sperm to use.
What is known already: Previous research has shown that normative expectations towards opposite-sex marriage and biological parenthood impose significant psychological burden on lesbians in China, where same-sex couples are not entitled to the rights to partnership/marriage, assisted reproductive technology (ART), and parenthood. Despite the legal barriers, online discussions on same-sex parenthood and commercial consultation services targeted at same-sex couples who want to travel overseas to use ART have emerged in recent years. While more lesbians have become parents of donor conceived children in Western developed countries, little is known about Chinese lesbians' reproductive experiences in the context of increasing reproductive transactions that transgress borders.
Study design, size, duration: In-depth, semi-structured interviews were conducted with 35 Chinese lesbians between July 2017 and June 2018 in Beijing, China. To better understand the context and social and clinical implications of global ART services for Chinese society, I also carried out participant observation by attending informal gatherings organised by the local lesbian community and public events targeted at (same-sex) individuals and couples who want to travel overseas to use ART and producing fieldnotes after the events.
Participants/materials, setting, methods: Participants were aged between 25 to 45. The majority were in their thirties. Each interview took around 2 hours and was audio-recorded and transcribed. The interview guide covered questions about their family beliefs, views on and/or experiences of donor conception, and perceived and actual difficulties in pursuing motherhood. With the assistance of NVivo (a qualitative data analysis software), I carried out thematic analysis of the interviews and fieldnotes to identify common patterns across the dataset.
Main results and the role of chance: Participants shared a belief that being biologically connected with their (prospective) children was, to varying extents, important to their families. They were at different stages of fertility decision-making, ranging from achieved motherhood (8 participants), actively planning to pursue motherhood (9), hesitation in taking action (11), and a lack of interest in or hope of becoming a mother (7). Almost all participants expressed that they did not prefer adoption and that they were reluctant to involve known sperm donors, who were considered a threat to their parental status. Rather, they were inclined to seek ART overseas in order to create their desired biological ties in a clinical setting. Issues including donor screening, desire for family resemblances, the status of biological and social mothers, and plans to purchase sperms from the same donor to conceive "siblings" were discussed in the interviews. It is evident that when deciding on whether to have a child and how to involve any third parties, participants tended to embrace the relational self and carefully balance individuals desires with familial and social expectations. The felt need to legitimise their relationships with donor conceived children imposes psychological burden on lesbian intended parents and discourages many from pursuing motherhood.
Limitations, reasons for caution: The findings of this qualitative study are not intended to be generalised to the whole lesbian population in China. Given the hidden nature of this population, my research, despite its small sample size, represents a significant step forward and calls for more quantitative and qualitative studies on lesbians' fertility health.
Wider implications of the findings: This study shows that lesbians' journeys to donor conception require not only medical and legal support but also psychosocial care that attends to one's perceived importance of biological ties and family beliefs. It sensitises healthcare professionals to the specific fertility-related psychosocial needs and concerns among lesbians in a family-centred context.
Trial registration number: not applicable

P-509 Patients' Uses and Experiences of Medically-Assisted Reproduction in France in 2020

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Study question: What are the uses and real-life experiences of patients currently treated in France in medically-assisted reproduction (MAR) centers?
Summary answer: One in four patients is in a situation of significant pain in relation to MAR; half are not optimistic about the outcome of their treatment.
What is known already: Internationally, work on the quality of life and impact of treatments has been completed in the field of endometriosis (Culley et al. 2017). In the field of MAR in France, one study underlines an important level of general well-being, with nearly 4 people out of 5 who say they are rather content (Coudrière et al. 2020). A better understanding of this result is however still lacking, especially in respect to whether patients actually believe in the success of their treatments, or how dependent satisfaction is on variables, like age, secondary infertility or the number of years engaged in fertility treatments.
Study design, size, duration: We conducted an online self-administered questionnaire by means of a two-step process: a first survey dedicated to patients' uses was carried out from January 15, 2020 to February 26, 2000; the following and complementary survey about real-life experience was conducted from March 3, 2020 to April 10, 2020. The initial sample included 1503 people before selecting relevant participants.
Participants/materials, setting, methods: The final sample of 967 patients targets patients that were enrolled in a French procreative medical center at the time of the study. Were excluded from the sample: ex-patients, patients who have not started treatment or are not followed in France, solo and homosexual care patient trajectories. The questionnaire was composed of 178 questions. In the questionnaire dealing with MAR uses, six fields were examined; in the case of real-life experience, eight themes were questioned.
Main results and the role of chance: The study shows that one in four patients suffers significantly in relation to MAR care; nearly half are pessimistic about treatment outcome and 65% claim that MAR prevents full enjoyment of everyday life. Further findings include: MAR is globally satisfying regarding the quality of care provided by practitioners and staff (80%), general administration of patients (77 to 87%), first encounters with staff and center (78 to 91%), quality of dialogue (81%), physical state of the premises (82 to 97%), continuum with professional activity (73%). Where centers/practitioners need to pay attention: one out of two patients wanted more means of communication; time management was an issue; 41% state that infertility prevents entrepreneurial outreach and general success; treatments strongly disrupt work for 83% and reduce performances for 79%; work rights, while known, were not respected in ¼ cases. Discoveries made: young women are not very positive, men are not well-identified in such medical trajectories, women's body image is completely transformed for 95%, 85.5% of women are depressed by seeing a pregnant woman, and 15% do not think about wanting a child daily.
Limitations, reasons for caution: This research, carried out online, was distributed mainly through a grassroot organization, potentially biasing representativeness. Also, the study finished at the start of the first French confinement. Previous studies show that the general context does not impact results, but this limitation cannot be ignored (Fisher, Bayhem, 2019).
Wider implications of the findings: These findings underline the need for a comprehensive conception of MAR, including more than medical support. It highlights anew the need to better take men into account in future research as well as people in their twenties as knowledge of these categories of people is still lacking.
Trial registration number: Not applicable

P-510 Psychological distress and quality of life in infertile women attending Infertility clinic at tertiary care center: a pilot study

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Study question: What are the levels of psychological distress (anxiety and depression) and quality of life faced by infertile couple presenting with or undergoing treatment for infertility?
Summary answer: The levels of psychological distress and quality of life seems to be affected more in women than their husbands and may require psychological intervention.
What is known already: Infertility is a biopsychosocial crisis which can cause psychological distress in the form of depression and anxiety, and can impair quality of life .It often has a stressful impact on relationships and can affect a couple's sex life. Most of the time these aspects are not explored and only medical and surgical treatment is offered depending on the cause. Assessing the psychological distress and quality of life contributes to decrease the stress and helps to improve the outcome of management by improving the relationship of the individual to achieve pregnancy. These women need psychological support, in the form of counselling.
Study design, size, duration: Cross-sectional study .100 infertile couples attending fertility clinics. Previous data indicate that the mean infertility specific QoL in infertile couples is 54.39 for females (nd 60.63 for males .Thus, a minimum sample size of 77 samples per group is needed to be able to reject the null hypothesis with probability 80% power. With a 30% dropout rate, the sample size is 100 samples per group. The study was conducted from Jan 2018 to June 2019.
Participants/materials, setting, methods: Infertile couples aged between 25 to 40years attending the Infertility outpatient clinic in OBG department, JIPMER, Pondicherry, India were recruited in to the study. Presence of a pre-existing major medical illness and presence of a major psychiatric illness were excluded from the study. After written consent, the severity of depression and anxiety was measured using the Hospitol Anxiety and Depression Scale (HADS Scale) and QoL was assessed with the Fertility Quality of Life (FertiQoL) questionnaire.
Main results and the role of chance: The average ages (SD) of men and women were 33.6 (4.29) and 31.3(4.03) years, respectively. Women reported higher levels of depression ($p<0.001$) and anxiety ($p<0.001$) as compared to their husbands. Depression and anxiety was noted more in women who were more than 32year age and prolonged duration of infertility. There was a significant association between depression, gender, duration of marriage and duration of infertility among the infertile couples.78% women felt that their attention and concentration were impaired and 63% responded that they cannot move towards their life goals . Around 72% and 60% felt drained out and lost, respectively. 57% women had fluctuant thoughts like hope and despair. More women felt were socially isolated and uncomfortable with social situations than men. 45% reported social pressure and 52% were felt angry because of fertility problem. Only 24% women were satisfied with support from friends and 37% of their family can understand them. Overall only 51% of the participants gave positive response to fertiqol questions. The total FertiQoL scores were significantly higher in the husbands than the wives ($p<0.001$). Poor Qol were significantly associated with male cause of infertility ($p=0.004$), primary infertility ($p=0.022$) and previous history of receiving multiple treatments ($p =0.020$).
Limitations, reasons for caution: The main limitation of the study is the cross-section study design which cannot detect exact cause of psychological distress and small sample size from single center which did not define entire population. Self reported questionairre was more subjective 5han objective which might be counfounding.
Wider implications of the findings: Couples going through infertility have a varying degree of emotional moods swinging between anxiety and depression impairing QoL.,women being affected more than men.Counselor who can empathize with the couple should form an integral part of the infertility team providing psychological intervention along with infertility treatment.
Trial registration number: NA

P-511 Characteristics of psychological services for couples undergoing ART treatment in Italy

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Study question: What are the characteristics of the psychological services for couples undergoing ART treatment in Italy?
Summary answer: In the 47% of ART clinics the psychologist is a stable member of the team whereas psychological services are used by 10-20% of couples. What is known already: ART treatments are complex and emotionally demanding for both men and women. During the procedure the couples often experience stress and several negative emotions. In Italy the Law 40/2004 establishes the guidelines for ART application. This law sustains the importance of offering psychological support to the people who undergo ART treatments at any stage of the medical procedure. However, in Italy there are no specific recommendations or protocols for psychological interventions in ART centres. Furthermore, there is little or no studies regarding the characteristics of the psychological services offered to those undergoing ART treatments.
Study design, size, duration: This observational study aims to explore the characteristics of psychological services in ART clinics. Therefore, the ART Italian National Register (National Health Institute) with the Department of Dynamic and Clinical Psychology and Health study (Sapienza University) proposed a national survay to the 306 Italian ART centres. The Medical Director of each centre was invited to participate by e-mail and to fill a questionnaire on an on-line platform. Data collection was between Jenuary to February 2020.
Participants/materials, setting, methods: The participation has been proposed to all the Italian ART centres (n=306). 35 centres have been previously excluded because participating in the first phase of the study regarding the questionnaire construction. Around the 40% of the centres participated in the study (n=113). The questionnaire contains 26 items with multiple response answers. It mainly explored: the presence of the psychologist in the ART centres, charachteristics of the intervention offered, percentage of couples using the psychological service.
Main results and the role of chance: In the 47% of ART centres the psychologist is a stable member of the team. The 38% of respondents reported that the psychologist works on call in the centre, the 18% reported that the psychologist is present in the centre 1-2 times a week whereas the 32% reported that the psychologist works in a private practice outside the centre. The reported percentages of couples using the psychological service are: 10-20% (69%), 20-30% (13%), 30-50% (5%), more than 50% (5%) and around the 100% (8%). The number of psychological sessions usually offered are: up to five (9%), four (13%), three (29%), two (27%), one (10%). Couples interventions represent the 73%, single patient intervention the 14%, whereas the 13% are group interventions. The 60% of respondent reported the absence of a protocol for psychological intervention in the ART centre whereas the 40% reported the presence of a protocol. In around the 50% of cases the psychological service represents an extra cost for the couple/individual. In the 60% of cases the psychologist is not involved in the team meeting, however the 87% of respondents reported that confronting with the psychologist on patients is percived as useful.
Limitations, reasons for caution: These results should be considered with cautions due to the rate of participation (around 40%). Furthermore, this is a national study and the charachteristics of psychological services offered in ART centres of different countries should be investigated.
Wider implications of the findings: In conclusion, the results show that psychological services in Italian ART centers are not yet fully operational and integrated in the ART procedure. All of this highlights the importance of further investigations with the aim to shared information to use to establish common protocols for psychological interventions in ART centres.
Trial registration number: not applicable

P-512 When COVID-19 pandemic seems to be a never-ending story: Fear and anxiety do not discourage infertility patients to seek for the treatment

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Study question: Do fear, anxiety and perceptions related to COVID-19 infection significantly impact infertility patients in seeking and continuing the treatment?
Summary answer: Despite fear and anxiety related to COVID-19 pandemic, 94.4% of infertility patients still decided to proceed with the infertility treatment. What is known already: It has been commonly acknowledged that infertility has a momentous impact on the psychological well-being of both partners. COVID-19 pandemic might significantly exacerbate the feeling of stress, anxiety and depression in patients with infertility problem. However, the concern that delaying the treatment may negatively impact the outcome has led to the continuation of infertility management during the pandemic era. In this context, social distancing and loss of social support could possibly be deepened, contributing to higher levels of distress. The patients may face a high level of uncertainty due to the inability to conceive and the spread of COVID-19 infection.
Study design, size, duration: This is a cross-sectional study utilising a questionnaire distributed to infertility patient in Jakarta. All the women gave their informed consent to collect and use their data for conducting this study. An anonymous electronic survey on Google Forms web application was e-mailed to infertile couples. The purpose of this survey was explained to all participants with a brief introduction. Survey participation was voluntary.
Participants/materials, setting, methods: This study utilising a questionnaire distributed to infertility patient in Jakarta. Patients were identified, and demographic data were collected between 1 September 2020 and 25 January 2021. The survey was distributed to a total of 178 patients with phone and email reminders.
Main results and the role of chance: A total of 178 patients were replied and filled the google form completely. The average age of the patients and the length of infertility were 32.6 ± 1.4 years and 60 ± 2.9 months, respectively. Most of the patients were University graduated (71.8%). Among all participants, 94.4% decided to proceed with the infertility treatment despite the COVID-19 pandemic. Ovulatory dysfunction was the most common type of infertility in women (46%). However, the percentage of the treatment delay due to hospital protocol or the patient's decision was 39.5%. The age and the length of infertility were significantly associated with higher anxiety levels ($p<0.05$). Patients knowledge regarding the COVID-19 infection was not significantly impact the level of anxiety ($p>0.05$). It was demonstrated that 37.3% of the patients preferred fresh compared to frozen embryo transfer despite COVID-19 pandemic, and 33.4% of the participants admitted that they had a financial problem during the COVID-19 pandemic. However, 25.9% of the patients decided to continue the treatment during the pandemic regardless of this financial problem.
Limitations, reasons for caution: The use of a cross-sectional study may lead to limited information regarding patients' characteristics prior to the COVID-19 pandemic. The length and implications of this pandemic are unpredictable that the outcomes of this study may not reflect sustained consequences of COVID-19 pandemic on psychological well being of women with infertility.
Wider implications of the findings: It is imperative to offer emotional support to reduce stress and concerns in women with infertility. As the willingness of infertility patients to continue the treatment despite pandemic COVID-19, the risks and consequences of COVID-19 infection should be addressed in managing patients with infertility during the pandemic era.
Trial registration number: Not applicable

P-513 Analysis of the extent of dropout-rates by extraction from cumulative live birth rates in IVF/ICSI: systematic review and meta-analysis

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Study question: The purpose of this systematic review is to calculate dropout-rates of IVF/ICSI treatment by analysing the published cumulative live birth rates of IVF/ICSI treatment.
Summary answer: One out of three patients stop their treatment after their first IVF/ICSI cycle and dropout-rates tend to increase per consecutive cycle.
What is known already: Cumulative live birth rates (CLBRs) have created the possibility to present realistic probabilities of having a live birth after IVF/ICSI treatment. However, it is noted that a significant percentage of the patients stop their treatment before having a child ("dropout"). Possible reasons and predicting factors for dropout of treatment are already extensively investigated.

However, only a few studies try to report about the incidence of dropout. Publications on CLBRs of large numbers of patients allow the extraction of dropout-rates. These rates will provide insight in the extent of the problem and could be used as a reference for interventional studies.

Study design, size, duration: Four databases (PubMed, The Cochrane Library, EMBASE, DoKS) were systematically searched from 1992 to December 2020. Search terms referred to "cumulative live birth" AND "ART/IVF/ICSI". No restrictions were made on the type or language of publication. Studies were included if they reported absolute numbers of patients and live births per consecutive complete IVF/ICSI cycle or per consecutive embryo transfer cycle, starting from the first IVF/ICSI cycle for each patient.

Participants/materials, setting, methods: Dropout-rates per cycle were calculated in two manners: "intrinsic dropout-rate" with all patients that started the particular IVF/ICSI cycle in the denominator, and "potential dropout-rate" with all patients who did not achieve a live birth after IVF/ICSI (and potentially could have started a consecutive cycle) in the denominator. Dropout-rates were analysed for consecutive complete cycles and consecutive embryo transfer cycles, because these two manners are used in reporting CLBRs, often related to the reimbursement policy.

Main results and the role of chance: This review included 29 studies and almost 800,000 patients from different countries and registries.

Regarding the patients who started their first IVF/ICSI cycle, trying to conceive their first child by IVF/ICSI, intrinsic dropout-rate was 33% (weighted average) after the first complete cycle, meaning they did not return for their second oocyte retrieval cycle. After the first embryo transfer cycle, intrinsic dropout-rate was 27% (weighted average), meaning those patients did not return for their next frozen-thawed embryo transfer cycle or for the next oocyte retrieval cycle. Regarding the patients who did not achieve a live birth after the first complete cycle, potential dropout-rate was 48% (weighted average), and 37% (weighted average) after the first embryo transfer cycle.

Both potential and intrinsic dropout-rates for both consecutive complete and embryo transfer cycles tended to increase with cycle number.

One study on second IVF/ICSI conceived children showed a potential dropout-rate after the first complete cycle of 29%. From studies on women >40 years of age, the potential dropout-rate after the first complete cycle was 45% (weighted average) and from studies with the uses of testicular sperm extraction, the potential dropout-rate after the first complete cycle was 34% (weighted average).

Limitations, reasons for caution: Our analysis was hampered by the different ways of reporting on CLBRs (complete cycles versus embryo transfer cycles), informative censoring, patients changing clinics and spontaneous pregnancies. Dropout-rates were potentially overestimated given that spontaneous pregnancies were not taken into account.

Wider implications of the findings: The extent of dropout in IVF/ICSI treatment is substantial and has an important impact on its effectiveness. Therefore, it is a challenge for fertility centers to try to keep patients longer on board, by taking into account the patients' preferences and managing their expectations.

Trial registration number: PROSPERO Registration number: CRD42020223512

POSTER VIEWING
REPRODUCTIVE (EPI)GENETICS

P-514 RAN-Translation in Fragile X associated Premature Ovarian Insufficiency (FXPOI): FMRpolyG as a predictive tool

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Study question: Does repeat-associated non-AUG (RAN) translation lead to accumulation of polyglycine- containing protein (FMRpolyG) in human lymphocytes and mural granulosa cells of *FMR1* premutation carriers?

Summary answer: Lymphocytes and granulosa cells from *FMR1* premutation carriers contain intracellular inclusions that stain positive for both FMRpolyG and ubiquitin. What is known already: Fragile-X-associated-Primary-Ovarian-Insufficiency (FXPOI) is characterized by oligo/amenorrhea and hypergonadotropic hypogonadism associated with the expansion of CGG-repeats in the 5'UTR of *FMR1*, called premutation (PM) (n: 55-200). Approximately 20% of women carrying a *FMR1*-premutation (PM) allele develop FXPOI. RAN-translation dependent on variable CGG-repeat length is hypothesized to cause FXPOI due to the production of a polyglycine-containing FMR1-protein, FMRpolyG. Recently, FMRpolyG inclusions were found in neuronal brain cells of FXTAS patients and stromal cells of the ovary of an FXPOI patient. Study design, size, duration: Lymphocytes and granulosa cells (GCs) from women with PM (6) and women without PM (10) (controls) were analyzed by immunofluorescence (IF) staining for the presence of inclusions positive for ubiquitin and FMRpolyG. Cell lysis and protein extraction samples were subjected to Fluorescent Western Blot (WB) analysis to detect FMRP and FMRpolyG

Participants/materials, setting, methods: Human GCs were obtained from follicular fluid after oocyte retrieval and lymphocytes were isolated from peripheral blood using Ficoll-Paque. Cells suspended in PBS were adhered to a glass-coverslip placed at the bottom of the 6-well culture plate, via gravity sedimentation. Adhered cells were fixed, IF staining for FMRpolyG and ubiquitin was performed and analyzed by fluorescence microscopy. Fluorescent WB was used to demonstrate the expression of FMRP, FMRpolyG in extracted protein from lymphocytes and GCs.

Main results and the role of chance: FMRP was successfully detected by fluorescence WB in both lymphocytes and GCs. FMRP is mainly present in cytoplasm and was expressed in greater amount in GCs than in leukocytes. Moreover, FMRP expression was significantly decreased in GCs from *FMR1*-PM compared with controls. Lymphocytes from PM-carriers and controls were immunostained for FMRpolyG and ubiquitin. In PM-carriers, FMRpolyG was present as aggregates, whereas in controls only a weak signal without inclusions was detectable. The expression pattern of FMRpolyG in GCs was similar to that in lymphocytes with a significant increase in PM-carriers. There, the FMRpolyG-aggregates additionally demonstrated as ubiquitin-positive inclusions. These may resemble the toxic potential of these protein fractions involved the ovarian damage in developing FXPOI.

Limitations, reasons for caution: More patients are needed to support the present findings. Further investigation into the possible consequences of these FMRpolyG-positive inclusions in PM-carriers is also advisable.

Wider implications of the findings: We found for the first time FMRpolyG-accumulation in lymphocytes and GCs from *FMR1*-PM-carriers in ubiquitin-positive inclusions. Future experiments evaluating consistency in more patients and elucidating the impact on fertility and prospective value for individual ovarian reserve are therefore in preparation.

Trial registration number: not applicable

P-515 Clinical outcome of mosaic-blastocyst transfer versus euploid-blastocyst transfer in single frozen blastocyst transfer cycles

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Study question: What is the clinical outcome of transferring a mosaic blastocyst versus a euploid blastocyst in single frozen blastocyst transfer (sFBT) cycles?

Summary answer: Single mosaic blastocyst transfer has similar clinical outcome to single euploid blastocyst transfer.

What is known already: Embryonic mosaicism occurs when there are two or more distinct cell lines found in preimplantation embryos derived from IVF. Data from recent studies show that mosaic blastocysts have the potential to implant and can result in healthy live births. As a result, patients now have the option of transferring mosaic blastocyst when they do not have any euploid blastocyst available for transfer. However, the clinical outcome of transferring mosaic blastocyst has not been definitively reported. Thus, a retrospective study was conducted to compare the clinical outcome of mosaic sFBT and euploid sFBT.

Study design, size, duration: A total of 602 patients underwent frozen blastocyst transfer in Alpha IVF from January to October 2019 and had their

blastocysts screened for aneuploidy. These patients were divided into 2 groups: 26 patients with mosaic blastocysts transferred (Group A, age ranged 19-44), and 576 patients with euploid blastocysts transferred (Group B, age ranged 21-44). The mean age of patients from Group A and B were 34.0 and 32.8 respectively (p>0.05).

Participants/materials, setting, methods: All samples had their DNA libraries constructed for sequencing using Next Generation Sequencing according to manufacturer's specification (IonTorrent, USA). All blastocysts were frozen for subsequent sFBT cycle (Cryotech, Japan). All thawed blastocysts for sFBT survived with morphologically intact inner cell mass and trophectoderm cells. The importance of antenatal confirmation of the fetal chromosome status was emphasized in patients from Group A. The clinical outcomes of both groups were analysed and compared.

Main results and the role of chance: No significant differences were seen in the clinical pregnancy and implantation rate of Group A and B (65.4% vs 63.0%; p>0.05). The miscarriage rate of Group A and B were 23.5% and 14.0% respectively. Albeit the higher miscarriage rate in Group A, there was no statistical significance between these two groups (p>0.05).

Group A was further divided into two subgroups, Subgroup A1: low risk mosaic blastocyst transfer; Subgroup A2: high risk mosaic blastocyst transfer. In the comparison of Group A subgroups, the clinical pregnancy and implantation of Group A1 is higher than Group A2 (76.9% vs 44.4%). In addition, the miscarriage rate of Group A1 and A2 were 23.1% and 0.0% respectively. Interestingly, there was no statistical significance in clinical pregnancy rate, implantation rate and miscarriage rate between these two subgroups.

Limitations, reasons for caution: This is a retrospective study and the sample size was comparatively smaller in the mosaic blastocyst transfer group than the euploid blastocyst transfer group. Further studies with a larger sample size should be carried out to ascertain the clinical outcome.

Wider implications of the findings: Single mosaic blastocyst transfer has similar clinical outcome to single euploid blastocyst transfer. Thus, mosaic blastocyst can be considered for transfer when no euploid blastocyst are available. Nevertheless, stringent antenatal surveillance for chromosomal abnormalities to confirm the chromosomal status of the fetus must be followed.

Trial registration number: Not applicable

P-516 Evidence for self-correction in preimplantation embryos by comparative molecular karyotyping of blastocoele fluid, trophectoderm and inner cell mass

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Study question: Does the molecular karyotype of the cell-free DNA (cfDNA) from the blastocyst fluid (BF) can predict the efficiency of self-correction of karyotype of preimplantation embryo?

Summary answer: Detection of aneuploidies in the BF potentially can point out on effective self-correction of blastocyst karyotype and consequently on high developmental potential of mosaic embryos.

What is known already: Correction of aneuploidies in the preimplantation embryos can be provided by several mechanisms, including apoptosis. The predominant death of aneuploid cells was demonstrated in mouse embryos (Bolton, 2016). A positive correlation was also shown between the concentration of cfDNA from the BF of human blastocyst and the morphology of the embryo, as well as between the activity of caspase-3 and the concentration of cfDNA (Rule, 2018). The incidence of failed amplification after WGA being significantly higher among euploid blastocysts (Magli, 2019). The capacity of abnormal cells extruding into the BF would be related to the embryo development potential (Gianaroli, 2019).

Study design, size, duration: This is a prospective observational study of thirty-one Day 5 human blastocysts. Cryopreserved blastocysts were received after treatment cycles at the IVF Center with informed consent obtained from couples. The average age of 15 women was 32.25±5 years. The morphological characteristics of blastocysts were estimated in accordance with the Gardner classification (Gardner, Schoolcraft, 1999). The procedure of BF aspiration and trophectoderm (TE) and ICM cells separation of the blastocysts was previously described (Tsuiko, 2018).

Participants/materials, setting, methods: WGA was performed by PicoPLEX kit (Rubicon Genomics, USA) or REPLI-g Mini kit (Qiagen) according to manufacturer's protocols. The DNA of the BF, ICM and TE were analyzed separately using cCGH, aCGH and NGS. SurePrint G3 Human CGH Microarrays (8x60K, Agilent Technologies) were used according to the manufacturer's recommendations. Image analysis was done using ISIS (v.5.5) (Metasystems) and Agilent CytoGenomics Software (v.3). VeriSeq™ PGS Kit - MiSeq® System (Illumina) was used for NGS.

Main results and the role of chance: Molecular karyotypes of all three samples - BF, ICM and TE, were obtained for 23 (74.2%) blastocysts. A correlation between the woman's age and the number of aneuploidies in cfDNA (p=0.0009) was found. A positive correlation may indicate that the number of aneuploidies in the embryonic cells increases with the age of a woman, however, the embryonic karyotype undergoes self-correcting through the elimination of aneuploid cells. It was noted that well-developing blastocysts (groups 4-5, according to Gardner's classification) had fewer aneuploidies in ICM (p=0.0141) and TE (p=0.0436). In contrast, there was a tendency to an increase in the number of aneuploidies in the BF during blastocysts transition from stage 3 to 5 (p=0.3542). We assessed the relationship between the number of aneuploidies in groups of blastocysts with different characteristics of ICM (groups "A" and "B" according to Gardner's classification). These groups significantly differ in the number of aneuploidies in cfDNA (p=0.0352), although the statistically significant differences between the number of aneuploidies in ICM (p=0.5992) and in TE (p=0.5934) was not detected. Thus, higher-quality embryos in terms of ICM morphology contain more abnormalities in the BF, since in this group the elimination of aneuploid cells is more efficient.

Limitations, reasons for caution: The number of embryos is limited in this study. More comprehensive studies are required to confirm the observed tendency.

Wider implications of the findings: Aneuploid cells elimination can be a cause of increasing cfDNA concentration in the BF, which may be a marker of the viability of mosaic embryos when it is necessary to decide on mosaic embryo transfer. This study was supported by the RFBR (15-04-08265) and by the RSF (20-74-00064).

Trial registration number: not applicable

P-517 The relationship between mitochondrial gene CYB (MT-CYB) single nucleotide polymorphisms and male infertility

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Study question: Do single nucleotide polymorphisms of the mitochondrial gene *CYB* (*MT-CYB*) affect male fertility?

Summary answer: there was a significant association between male fertility and rs527236194, rs28357373, and rs41504845 single nucleotide polymorphisms of the mitochondrial gene *CYB* (*MT-CYB*).

What is known already: Male infertility can be occurred as a result of various factors. However, genetic factors are detected in 15% of male infertility cases and can be classified into two groups: chromosomal abnormalities and single gene mutations. Sperm mitochondrial DNA alterations may have serious effects on spermatogenesis, sperm motility and the ability of sperm to fertilize the oocyte. Mutations of the *MT-CYB* gene might lead to various disorders and deficiencies specially in complex III which might interrupt in the ATP production process. Study design, size, duration: 111 semen samples were included in this prospectively designed study which carried out between 2017 and August 2019. Participants/materials, setting, methods: This study carried out at the Department of Obstetrics and Gynecology at Saarland University, Germany. Samples were divided into 67 subfertile "cases" and 44 fertile "control" groups.

After preparation of semen samples by density gradient centrifugation, nuclear and mitochondrial DNA (MT-DNA) was extracted using QIAamp DNA Mini Kit from QIAGEN. Thereafter, the MT-DNA was amplified using REPLI-g Mitochondrial DNA Kit from QIAGEN, followed by PCR and Sanger sequencing steps.

Main results and the role of chance: A total of 13 single nucleotide polymorphisms (SNPs) in the *MT-CYB* gene in each of the case and control groups were detected. Eight SNPs were non-synonymous variants including: rs2853508, rs28357685, rs41518645, rs2853507, rs28357376, rs35070048, rs2853506, and rs28660155 and five SNPs were synonymous variants: rs527236194, rs28357373, rs28357369, rs41504845, and rs2854124.Among these SNPs, three of them showed a significant difference in the genotype's frequency test between sub-fertile and fertile groups: rs527236194 (T15784C; P=0.0005), rs28357373 (T15629C; P=0.0439), and rs41504845 (C15833T; P=0.0038). For the allele's frequency test, two SNPs were significant: rs527236194 (T15784C; P=0.0014) and rs41504845 (C15833T; P=0.0147).
Limitations, reasons for caution: The study population size
Wider implications of the findings: A larger prospective study will be required to confirm the associations between these mitochondrial gene polymorphisms in *MT-CYB* (rs527236194, rs28357373, rs41504845) and male infertility and to clarify the definite effect of the mitochondrial genetic variations on male infertility.
Trial registration number: not applicable

P-518 Assessment of mitochondrial DNA viability ratio in day-4 biopsied embryos as an add-in to select euploid embryos for single embryo transfer

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Study question: Is mitochondrial DNA viability ratio of day-4 biopsied embryos associated with embryo implantation potential?
Summary answer: The mitochondrial DNA viability ratio is significantly higher in embryos that implant. The score might help to select euploid embryos for single embryo transfer.
What is known already: Embryo euploidy is a critical factor for successful pregnancy outcomes. However, transfer of euploid embryos does not invariably result in implantation, thus indicating that other factors may play a role. Metabolic rates and adenosine triphosphate content vary significantly in oocytes and embryos and might affect embryo viability. Embryo function, indirectly measured by mitochondrial DNA viability ratio (mtV) has emerged as a potential quantitative biomarker for embryonic selection before the transfer, but clinical data remains limited. The purpose of this study is to characterize and compare mtV in euploidy day 4 embryos.
Study design, size, duration: Retrospective cohort study carried out between Jan. 2017 to Jan. 2020, involving 75 infertile couples undergoing IVF-ICSI with PGT-A and single embryo transfer (SET) of day 4 euploid embryos.
Participants/materials, setting, methods: We compared the mtV ratios of 34 non-pregnant patients with those of 41 patients who achieved clinical pregnancy after SET. The mtV ratio was obtained from a cohort of 75 euploidy embryos. The embryos were biopsied 80-85 hours post–ICSI and subjected to next-generation sequencing (NGS). The mtV was determined using Multiple of Mean (MoM) values, obtained by dividing the mtV ratio of individual embryos by the mean mtV value of all implanted embryos.
Main results and the role of chance: The mean mtV ratio (1.51; 95% confidence interval [CI] 1.25-1.77) of non-pregnant patients was significantly lower than those of pregnancy counterparts (2.5; 95% CI 1.82-2.68; p<0.01). At a 0.5 MoM cutoff, the sensitivity and specificity of mtV ratio to discriminate between implanted embryos versus non-implanted embryos were 35.3% and 78.2%, respectively., with a positive predictive value (PPV) of 41.4%.
Limitations, reasons for caution: Our study is limited by the small sample size and lack of stratification by causes of female/male infertility. Endometrial receptivity issues, which could have contributed to implantation failure, was not evaluated.
Wider implications of the findings: Assessment of mtV ratio could provide additional prognostic information for selecting euploid embryos for transfer in SET programs. Further research is warranted to establish the clinical utility of routine application of mtV evaluation in PGT programs.
Trial registration number: N/A

P-519 Investigation of embryo chromosomal constitution and live birth rate after vitrified-warmed euploid single blastocyst transfer across ranges of maternal body-mass-index

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Study question: Does maternal body-mass-index (BMI) associate with blastocysts' chromosomal constitution and clinical outcomes in infertile patients undergoing preimplantation genetic testing for aneuploidies (PGT-A)?
Summary answer: A higher euploidy rate per biopsied blastocyst was reported among underweight women. Overweight women were instead subject to higher miscarriage (MR) and lower live-birth-rates (LBR).
What is known already: Different studies in the literature revealed an association between BMI and infertility, suggesting a J-shaped relationship: both underweight and overweight women can suffer from infertility issues. Even if IVF might increase the success rate in both these categories of patients, it seems insufficient *per se* to overcome the complex and multifactorial fertility impairment derived from unbalanced nutritional intakes. Miscarriage, in particular, is common in both underweight and overweight women. However, most of the literature is based on chromosomally-untested embryos. Study design, size, duration: Retrospective observational study. Only the first IVF cycle with ≥1 biopsied blastocyst from each woman was included. The primary outcome was the association between maternal BMI (underweight, BMI<18.5, n=160; normal-weight, BMI=18-25, N=1392; overweight, BMI>25, N=259) and the mean euploidy rate per cohort of biopsied blastocysts (m-ER). The secondary outcomes were the association between maternal BMI with clinical (mainly MR and LBR), gestational and perinatal outcomes after first vitrified-warmed single euploid blastocyst transfers.
Participants/materials, setting, methods: We included 1811 women undergoing PGT-A at a private IVF center between April-2013 and March-2020. The secondary outcomes were investigated on 1125 first vitrified-warmed single euploid blastocyst transfers from all patients obtaining ≥1 transferable blastocyst.

Age	# zygotes mean	# cleavage-stage embryos mean (% per 2pn)	# blastocysts mean (% per 2pn)	# biopsy-quality blastocysts mean (% per 2pn)	# euploid blastocysts mean (% per 2pn)
<35 N=15	2.40	2.40 (100.0)	2.00 (83.3)	1.53 (63.8)	0.67 (27.9)
35-37 N=15	2.40	2.40 (100.0)	1.67 (69.6)	1.20 (50.0)	0.60 (25.0)
38-40 N=32	2.31	2.22 (96.1)	1.59 (68.8)	1.03 (44.6)	0.25 (10.8)
41-42 N=11	2.27	2.27 (100.0)	1.18 (52.0)	0.73 (32.2)	0.09 (4.0)
>42 N=12	2.50	2.50 (100.0)	1.67 (66.8)	1.17 (46.8)	0.00 (0.0)

Only ICSI with ejaculated sperm and continuous culture in standard incubators were performed. Logistic regressions were conducted to identify putative confounders and adjust the results accordingly.

Main results and the role of chance: Except for a lower maternal age among underweight women (38.3±3.1 versus 38.9±3.4 yr, p<0.01) and higher among overweight ones (39.3±3.6 yr, p=0.04), no difference was reported with respect to normal-weight women in terms of duration of infertility, hormonal levels, main cause of infertility, sperm quality, and reproductive history. The mean number of biopsied blastocysts was ~3 in all groups. The m-ER shows a decreasing trend as the maternal BMI increases between 17 and 22-23, to then plateau. In fact, a significant difference was reported between underweight (50.8%±36.4%) and normal-weight women (41.4%±37.5%, p<0.01). A linear regression adjusted for maternal age confirmed this moderate association between increasing BMI and m-ER (unstandardized-coefficient-B -0.6%, 95%CI:-1.1% to -0.1%, p=0.02).

Morphological quality and day of full-blastulation among transferred euploid blastocysts was similar in the three groups. Overweight women showed higher MR per pregnancy (N=20/75, 26.7%, 95%CI:17.4%-38.3% versus N=67/461, 14.5%, 95%CI:11.5%-18.2%; OR 2.0, 95%CI:1.1-3.6, p=0.01) and lower LBR per transfer (N=55/154, 35.7%, 95%CI:28.3%-43.8% versus N=388/859, 45.2%, 95%CI:41.8%-48.6%; OR adjusted for euploid blastocysts' features 0.67, 95%CI:0.46-0.96, p=0.03). Clinical outcomes were instead similar among underweight and normal-weight women. All gestational and perinatal outcomes were comparable in the tree groups.

Limitations, reasons for caution: Our study is limited by its retrospective nature, and the fact that maternal BMI was measured only before oocyte retrieval and not before embryo transfer. Moreover, the reduced sample size did not allow for further relevant sub-analyses among solely obese women.

Wider implications of the findings: When possible nutritional/lifestyle modifications should be encouraged to adjust maternal BMI before IVF. Overweight patients should be especially informed of their higher risk for miscarriage. Yet, BMI is just a gross marker, future studies based on body fat localization and percentage (e.g. by bioelectrical impedance analyses) are desirable.

Trial registration number: None

P-520 Live birth rate with a euploid embryo is the same irrespective of the number of oocyte retrievals undertaken to produce a euploid embryo

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Study question: Does a euploid embryo from one ovarian stimulation lead to the same live birth rate as a euploid embryo arising from multiple ovarian stimulations?

Summary answer: The live birth rate of a euploid embryo transferred is comparable irrespective of the number of ovarian stimulations required.

What is known already: Embryo transfer of a euploid embryo leads to a high live birth rate. Women with low ovarian reserve or poor responders may not have a euploid embryo from one cycle of ovarian stimulation and can be discouraged from undergoing preimplantation genetic testing for aneuploidy (PGT-A). Embryo batching from multiple cycles offers such patients a potential solution to increase their chance of achieving a euploid embryo.

Study design, size, duration: A retrospective analysis of 506 cycles of single euploid frozen embryo transfers (FET) from January 2015 to March 2020 was carried out. The indication for PGT-A was advanced maternal age, recurrent miscarriages or repetitive IVF failures. Only the first single euploid FETs per patient were included.

Participants/materials, setting, methods: Group A (N=323) included women who had a normal ovarian reserve and only one cycle of ovarian stimulation before the FET, whilst Group B (N=183) had low ovarian reserve or previous poor ovarian response requiring 2 or more cycles of ovarian stimulation. All embryos were biopsied at the blastocyst stage and subjected to a-CGH or NGS. Univariate statistical analysis with Chi-square or Wilcox Man U as required and multivariate logistic regression was performed with SPSS.

Main results and the role of chance: Group A and Group B were comparable in terms of BMI (average 22.3 vs 22.6), sperm origin, number of blastocysts biopsied (median N=6, range 4-8), day 5 vs day 6 biopsy (day 5, 81.1% vs 75.4%, p=0.130) and whether only one euploid embryo was available (42% vs 34%; p=0.103). There was a significant difference in the number of eggs retrieved per cycle between the two groups (median 15 vs 9, p<0.001), the total number of eggs retrieved (median 15 vs 20, p<0.001) and whether a top-quality embryo was transferred (38% vs 25%, p=0.026). Pregnancy rate, live birth rate and pregnancy loss was equivalent for both groups: 69.3% (224/323) vs 63.9% (178/183) (p=0.212), 57.6% (186/323) vs 57.4% (105/183) (p=0.096) and 17.0% (38/224) vs 10.3% (12/117) (p=0.964), respectively.

Multivariate logistic regression was performed to ascertain the effect of the treatment variables on the live birth rate. The number of oocyte collections was not a significant predictive factor (OR 1.19, 95% CI 0.72 - 1.96, p=0.491), whilst an embryo biopsy performed on day 5 vs day 6, increased significantly the live birth rate (OR 2.58, 95% CI 1.61 - 4.13, p<0.001).

Limitations, reasons for caution: The main limitation of this study is that it is retrospective, single centre and therefore vulnerable to confounding factors and bias.

Wider implications of the findings: These results can be used to counsel and reassure women with poor response embarking on embryo batching and PGT-A that should a euploid embryo become available, their chance of success is unaffected by the number of cycles they undertake albeit the physical, emotional and financial burden of multiple ovarian stimulations

Trial registration number: not applicable

P-521 Association between maternal age and euploid blastocyst availability in cycles with less than four two-pronucleate zygotes

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Study question: For patients with less than four two-pronucleate (2pn) zygotes, is there an age-cutoff above which preimplantation genetic diagnosis for aneuploidy (PGT-A) is futile?

Summary answer: Women over 40y with less than four 2pn zygotes should consider transfer of a day 3 embryo over culture to blastocyst with PGT-A.

What is known already: During a typical IVF cycle, there is unavoidable attrition from oocytes retrieved, to embryos obtained, to blastocysts formed such that some patients, particularly those with advanced age or poor ovarian response, may not have blastocysts available to biopsy. While randomized trials have shown improved pregnancy rates with the use of PGT-A in patients of advancing age, these trials primarily included patients with good ovarian reserve and multiple blastocysts available. The optimal age group within poor responders who would benefit most from PGT-A has yet to be determined.

Study design, size, duration: This was a retrospective cohort study of all fresh autologous IVF or IVF/ICSI cycles in which PGT-A was planned from 1/2012 to 3/2020. Only patients with less than four 2pn zygotes were included. A total of 85 cycles from 75 patients were analyzed.

Participants/materials, setting, methods: Number of cleavage-stage embryos, blastocysts, biopsy-quality blastocysts and euploid embryos were assessed, after stratification by age. Adjusted relative risks (aRR) and 95% confidence intervals (CI) were calculated adjusting for BMI, AMH, FSH, stimulation protocol, and ICSI. Poisson regression was used for counts. Generalized estimating equations were used to account for patients contributing multiple cycles.

Main results and the role of chance: There were no differences in number of 2pn zygotes (p=0.98) or cleavage stage embryos (p=0.94) across age groups. Patients aged 41-42y had a significantly lower number of blastocysts (1.18 vs. 2.00; aRR 0.59 95%CI: 0.37-0.95) and biopsy-quality blastocysts (0.73 vs. 1.53; aRR 0.50 95% CI: 0.26-0.98) compared to patients <35y.These patients also had fewer euploid embryos available (0.09 vs 0.67), although the difference was not significant in the adjusted model (aRR 0.14 95% CI: 0.01-1.57). None of the patients >42y had euploid blastocysts. When considering the mean and three standard deviations (0.09 [SD 0.3]), 99.7% of patients over 40y have no euploid embryo available for transfer.

Limitations, reasons for caution: This study was retrospective in nature and limited by small sample sizes when patients were stratified by age. A prospective randomized trial of patients with less than four 2pn zygotes to day 3

fresh embryo transfer vs PGT-A frozen embryo transfer is needed to confirm these findings.
Wider implications of the findings: Patients over 40y with less than four 2pn zygotes are at high risk of having no euploid blastocysts. While the literature demonstrates higher live birth rates with the use of PGT-A in women of advancing age, this is inconsequential if there is no embryo available to transfer.
Trial registration number: not applicable

P-522 Cervical secretion methylation profile is associated with the success of frozen-thawed embryo transfer - a proof-of-concept study

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Study question: Is cervical secretion gene methylation profile different between receptive and non-receptive endometrium and associated with implantation outcome in frozen-embryo transfer (FET) cycle?
Summary answer: The combination of candidate genes methylation profiles obtained from cervical secretion showed significant associations with pregnancy outcomes.
What is known already: Implantation failure remains a black box in reproductive medicine, and the exact mechanism of how endometrial receptivity is regulated is still unknown. Epigenetic modifications play a role in the gene expression pattern and may alter the endometrial receptivity in the human endometrium. Cervical secretion containing various implantation-related cytokines, and the gene methylation change can be used as a non-invasive molecular source that reflects the endometrium condition.
Study design, size, duration: In this retrospective case-control study, sixty-two women who entered the FET cycle (30 pregnant and 32 non-pregnant women) were enrolled.
Participants/materials, setting, methods: Cervical secretion was collected before embryo transfer from women enrolled in multicenter university-affiliated reproductive units. The DNA methylation status of six candidate genes was measured using quantitative methylation-specific PCR (qMSP). The correlation between methylation change and the pregnancy outcome was analyzed.
Main results and the role of chance: The candidate genes were selected from that associated with implantation with literature review and the original genome-wide DNA methylation data from NCBI GEO DataSets (GSE90060) which processed using bioinformatics analysis. Six candidate genes whose CpG-level methylation analysis with -value statistically higher in receptive endometrium than in a pre-receptive endometrium were selected. All six candidate genes showed different degrees of correlation with the pregnancy outcomes. Among them, *PRKAG2* methylation changes showed the highest correlation with the pregnancy outcome. A logistic regression model was used to evaluate the performance of a single gene or a combination of genes for implantation prediction. The results showed a statistically significant association between the methylation status of a combination of genes (*PRKAG2*, *KRS1*, *HAND2*) and the pregnancy outcome ($p = 0.008$), resulting in an optimal AUC of 0.7 (95% CI: 0.57 - 0.81) for implantation prediction.
Limitations, reasons for caution: The results obtained from a relatively small cohort size. A larger study and further comprehensive methylome investigations are warranted.
Wider implications of the findings: This study is the first proof-of-concept study that cervical secretion methylation profile is associated with implantation outcome in a FET cycle, and showed potential as a non-invasive method for implantation prediction.
Trial registration number: non applicable

P-523 Whole-chromosome aneuploidies revealed by transcriptome of trophectoderm biopsied from human pre-implantation blastocyst

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Study question: Whether mRNA transcriptome of biopsied trophectoderm (TE) in human pre-implantation blastocyst can predict embryo karyotype?
Summary answer: mRNA transcriptome of biopsied TE can precisely predict whole-chromosome aneuploidies but not mosaicism or segmental aneuploidies.
What is known already: Karyotype of human pre-implantation blastocyst is well recognized by PGT-A. However, genome can't demonstrate gene expression level which might infer the development potential of euploidy. Transcriptome of blastocyst by singe-cell RNA-seq has revealed the lineage segregation of human pre-implantation blastocyst. It is not known whether transcriptome of biopsied TE used in PGT-A can infer the karyotype of human pre-implantation blastocyst.
Study design, size, duration: A total of 74 TE samples were biopsied from 26 blastocysts which were donated from patients who underwent PGT at our Reproductive Medicine Center. All of these embryos have been previously diagnosed as aneuploidies (n=19) or euploidies (n=7) with monogenic disorder.
Participants/materials, setting, methods: The DNA and mRNA of all biopsied TEs were separated independently using a modified oligo-dT bead capture, followed by PGT-A of DNA and smart2-sequencing of mRNA (G&T-seq). Karyotype of biopsied TEs were confirmed with PGT-A performed in MiSeq system (Illumina) in our PGT laboratory with the use of next-generation sequencing. Data of transcriptome was analyzed using Rstudio and R package InferCNV to predict aneuploidies by referring to euploidies which were inferred with corresponding PGT-A results.
Main results and the role of chance: In human pre-implantation blastocyst, all whole-chromosome aneuploidies could be inferred by transcriptome of biopsied TE, which were consistent with PGT-A result. But chromosomal mosaicism or segmental aneuploidies were hard to be predicted precisely by transcriptome of TE.
Limitations, reasons for caution: The main limitation of this study lies in the inability to retrieve the exact copy number variations from mRNA transcription. Gene expression is in a great imbalance in such an early development of human pre-implantation blastocyst.
Wider implications of the findings: Our data suggest that mRNA transcriptome is enough for prediction of whole-chromosome aneuploidies. The method and value for predicting mosaicism and segmental aneuploidies by transcriptome should be further investigated.
Trial registration number: not applicable

P-524 Is polycystic ovary syndrome really a risk factor for embryonic chromosomal aberrations? A multicenter retrospective cohort study

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Study question: Does the rate of embryonic chromosomal aberrations increase in the setting of PCOS independent of maternal age and BMI?
Summary answer: Controlling for maternal age and BMI, embryonic chromosomal aberration rate was not significantly different with controlled women undergoing preimplantation genetic testing for monogenic defects (PGT-M).
What is known already: It has been reported that women with PCOS have higher risk of early spontaneous pregnancy loss, and it is well known that embryonic chromosomal abnormalities play an important role as a direct factor. However, whether PCOS women have increased risk of embryonic chromosomal aberrations remains inconclusive.
Study design, size, duration: A multicenter retrospective cohort study was undertaken examining the incidence of chromosomal abnormalities in blastocysts using next-generation sequencing (NGS) for women undergoing PGT-M with and without PCOS (1398 PGT cycles, 5577 blastocysts) from 3

university-affiliated IVF centers between 2015 and 2019. Participants/materials, setting, methods: The blastocyst formation rate and the incidence of chromosomal abnormalities were compared between 163 PCOS women and 1235 non-PCOS women. Main results and the role of chance: Stratification analysis by maternal age with matched BMI showed no differences with regard to blastocyst formation rates for women with and without PCOS aged 20-29y (55.0% vs. 58.5%), 30-34y (54.7% vs. 58.9%) and >35y (56.7% vs. 52.4%), *P*>0.05. The total embryonic chromosomal aberration rates for women aged 20-29y, 30-34y and >35y with and without PCOS were were also comparable, which were respectively 121/331 (36.4%) vs. 496/1209 (41.0%); 89/251 (35.5%) vs. 903/2175 (41.5%) and 72/130 (55.4%) vs. 789/1481 (53.3%), *P*>0.05. Multivariate regression showed that controlling for maternal age and BMI, PCOS were not an independent risk factor for embryonic chromosomal abnormalities (OR = 0.89, 95% CI = 0.62 ~ 1.35, *P* = 0.73).

Limitations, reasons for caution: The study is mainly limited by its retrospective design and relatively smaller sample size for PCOS group which carries inherent potential for bias (i.e. misclassification and errors due to inadequate clinical notes).

Wider implications of the findings: Our results indicated that chromosomal abnormalities might not be the most important causal factor for the increased risk of early pregnancy loss for women with PCOS. By contrary, the non-chromosomal embryonic aberrations and/or maternal intrauterine factors could play more important role and needs to be clarified

Trial registration number: not applicable'

P-525 Analysis of segregation patterns of trivalent structure and the effect on genome stability in Robertsonian translocation carriers

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Study question: What are the factors that affect the separation pattern of Robertsonian translocation trivalent, and whether the structure of the trivalent affected the chromosome stability?

Summary answer: The meiotic segregation modes can be affected by the carrier's sex and special chromosome, and a trivalent structure can affect the stability of the genome.

What is known already: Robertson translocation occurs when two proximal acrocentric chromosomes fuse at the centromere, and forms a trivalent structure during meiosis. This structure will affect the fertility of Robertsonian translocation carriers, and may destroy the stability of the genome by affecting the separation of other chromosomes, which is called Inter-Chromosomal Effect (ICE). Previous research have confirmed that the use of PGT in Robertsonian translocation carriers can effectively reduce abortion and increase live birth. But some studies dispute this conclusion and the existence of ICE. However, there is no large data study to verify these controversies.

Study design, size, duration: PGT results of 928 oocyte retrieval cycles in 763 couples(one of the couples is a Robertsonian translocation carrier) were analysied from December 2012 to June 2020. A total of 1492 couples who received PGT-A were collected as control group, and matched according to age and testing time stage. The study was approved by the ethics committee(LL-SC-SG-2006-008 and LL-SC-SG-2014-016).

Participants/materials, setting, methods: Cytogenetic analysis was performed using GTG standard method (trypsin and GiemsaG banding) to analyze the chromosomes of peripheral blood lymphocytes. Blastocysts obtained by standard IVF procedure were biopsied on the 5th or 6th morning after fertilization, and the trophoblast cells were amplified by PicoPLEX whole genome amplification kit (Rubicon Genology) or Repli-g Single Cell Kit(Qiagen). PGT-SR was performed using SNP array or NGS as previously described.

Main results and the role of chance: In this study, a total of 3423 blastocysts from 763 couples were analysed using SNP-array or NGS. Among them, the rate of alternate segregation of male Robertsonian translocation carriers was significantly higher than that in female carriers (82.26% vs 59.96%, P < 0.001), and meiotic segregation modes could be affected by the special chromosome such as 13 in female(P=0.042) and 15 in male(P=0.045) involved. A trivalent structure can affect the stability of the genome during mitosis, which is associated with an increase in the propotion of chromosome mosaic compared with the PGT-A control group(1.18% vs 0.53%, P < 0.01). In addition, we found an interesting phenomenon: in the meiotic segregation of female Robertsonian translocation carriers associated with chromosomes 21 and 22, the chromosome 21 or 22 of the two chromosomes involved in translocation are more likely to be abnormal, and according to our results, the effect of chromosome 21 seems to be greater.

Limitations, reasons for caution: (1) Limitations of retrospective analysis; (2) The results are not fully representative of the general population; (3) PGT-A patients always had repeated implantation failure or recurrent aboration, which may cause deviation to the results.

Wider implications of the findings: This study analyzed the influencing factors of the separation patterns of trivalent, and verified the existence of ICE. This suggest that PGT-SR can have a better outcome in patients with Robertsonian translocation, especially in male carriers. These results will provide carrier couple with more appropriate genetic counseling.

Trial registration number: no

P-526 Incidence of mosaic embryos in day-6 blastocysts, may late blastulation predispose to mosaicism?

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Study question: May the mosaicism ratio be influenced by the time of blastulation in preimplantation genetic testing for aneuploidies (PGT-A)?

Summary answer: The mosaicism ratio is significantly higher in day-6 blastocysts when compared to day-5 when transferable embryos are considered *only* (euploids and mosaics).

What is known already: Conventionally, PGT-A has classified preimplantation embryos as either euploid or aneuploid. Yet, a major improvement in PGT-A methodology, with the introduction of high sensitivity diagnostic Next Generation Sequencing (NGS) technology, has allowed the identification of a third embryo category: the mosaic (Coll et al., 2021). Embryonic mosaicism can be defined as the presence of karyotypically distinct cell lines within an embryo and can be detected by NGS at a rate between 20-80%. In the absence of euploid embryos, mosaic embryos, when transferred, have been shown to deliver healthy live births (PGDIS, 2019).

Study design, size, duration: This retrospective study was based on 9828 trophectoderm biopsies performed in a single ART clinic between January 2017 and October 2020 for PGT-A cycles with more than one blastocyst. PGT-A cycles with only one blastocyst were excluded because in these cycles' day-5/day-6 biopsy percentage cannot be calculated. A total number of 8398 and 1430 blastocysts were biopsied on day-5 and day-6, respectively for PGT-A by ReproSeq on Ion Torrent S5 (Thermo Fisher Scientific).

Participants/materials, setting, methods: Three categories were defined in the PGT-A group with >1 blastocyst biopsied to compare the rate of mosaicism: C1:cycles in which blastocysts were only biopsied on day-5 (n=1872), C2:99-60% of blastocysts were biopsied on day-5 (n=483) and C3:0-60% of blastocysts biopsied on day-5 (n=411). The mean female age (C1:36.0±5.2; C2:35.7±4.8; C3:37.1±4.9) and metaphase II oocytes punctured (C1:9.8±6.5; C2:10.4±5.2; C3:8.4±5.4) were similar and statistically non-significant in all groups. T-test and Chi-square tests were used where appropriate.

Main results and the role of chance: Overall, from the blastocysts biopsied on day-5 and 6, 35.4% and 25.5% were euploid, 13.7% and 14.7% were mosaic, 50.9% and 59.8% were aneuploid, respectively (p<0.0001, p=0.32, p<0.0001), the mosaicism rate being not statistically different. However, when *only* transferable blastocysts (euploids and mosaics) were considered (aneuploids being discarded), the rate of mosaic embryos was significantly higher in day-6 when compared to day-5 blastocysts (36.6% vs. 28.0%; p<0.0001). Morphological blastocyst grading was then investigated: from the day-5 and 6 blastocysts biopsied, 51.5% and 30.8% were of top-quality and 48.5% and 69.2% were of good-quality, respectively. Looking deeper into the categories defined, the

euploidy rate was found to be higher in C1 (35.1%) when compared to C2 (34.9%) and C3 (27.7%) (p<0.0001). The mosaic embryo rate was found to be non-significant when all blastocysts (euploids, aneuploids, mosaics) were considered (C1: 13.7%; C2: 14.1%; C3: 14.2%; p=0.6492). However, when transferable blastocysts were considered (euploids and mosaics only), the mosaic embryo rate was significantly higher in C3 (33.9%) when compared to C1 (28.1%) and C2 (28.8%) (p=0.02). For morphological blastocyst grading, regardless of blastulation day, mosaicism was higher for good-quality embryos both when all biopsies and only transferrable embryos were considered (p=0.0018, p<0.0001, respectively).

Limitations, reasons for caution: This study focused on blastocyst formation day and morphological blastocyst grading. Extrinsic factors have also been reported to induce mosaicism: ovarian stimulation, culture media, laboratory and culture conditions, technical issues during the biopsy and sample processing (Munné and Wells, 2017; Katz-Jaffe et al., 2018, Fragouli et al., 2010, 2019).

Wider implications of the findings: Mitotic errors during cleavage stage causing mosaicism may lead to lower morphological grade and late blastulation as some cells in those embryos are not diploid thus leading to higher mosaicism for blastocysts that reach blastulation on day6 and/or yield only good-quality embryos.

Trial registration number: not applicable

P-527 Embryonic cell-free DNA (cfDNA) in spent culture medium for aneuploidy screening and its concordance with trophoectoderm biopsy in PGT-A cycles

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Study question: Is embryonic cfDNA detectable in blastocyst spent culture medium; can its analysis via Next Generation Sequencing compare with trophoctoderm biopsy results of the respective embryos?

Summary answer: Embryonic cfDNA is detected in blastocyst spent culture medium (BCM) and gives comparable aneuploidy rates with trophoectoderm biopsy in PGT-A cycles.

What is known already: Currently, PGT- A involves the use of invasive techniques to obtain embryonic DNA, with significant technical and ethical limitations. Recently, spent culture medium (SCM) has been proposed as an alternative source of embryonic DNA. Studies have reported the detection of cfDNA in SCM and highlighted the diagnostic potential of non invasive PGT (niPGT) for assessing the genetic status of preimplantation embryos. Moreover, invasive PGT-A can lead to genetic misdiagnosis in case of mosaic embryos, while niPGT-A may be more represantative of the whole embryonic chromosome status. However, the reliability of this approach for clinical applications needs to be further determined.

Study design, size, duration: These are preliminary data from an observational study conducted in the period 2019-2020. 40 embryos from 13 patients, undergoing PGT-A, were analyzed. Trophoectoderm biopsies (TEB) and respective SCMs from individually cultured embryos were analyzed by Next Generation Sequencing NGS. The results from trophoectoderm biopsies and SCMs of the respective embryos were compared.

Participants/materials, setting, methods: The embryos were cultured individually in 10μl drops, from day 3 to the blastocyst stage (day5/6). On day 5/6, TEB was performed and the corresponding BCM was collected and stored at –80°C, until analysed with NGS. Data were analysed with McNemar's test and ROC analysis. The results were considered significant when P<0.05. 95% Confidence intervals (95%CI) were calculated.

Main results and the role of chance: The amplification rate, for embryonic cfDNA from BCM samples collected from embryos cultured for 48-72 hours after day 3, was 100%. DNA concentration in each sample after whole genome amplification (WGA) ranged between 2500-30000 ng/ml for TEB and 2000-20400 ng/ml for BCM. Respective blank medium negative controls associated with each sample that underwent WGA showed no amplification in all cases. The trophoectoderm biopsy showed aneuploidy at a percentage of 61% (95% CI: 43-78%), vs. BCM aneuploidy at a percentage of 55% (95% CI: 37-72%). The overall agreement BCM vs. TE biopsy, from samples taken from the same embryo, was 27/33, 81.8% (95% CI: 68-96%). McNemar test: p=0.687, non-significant. The aneuploidy agreement was 88.9% (sensitivity) and the euploidy agreement was 73.3% (specificity). In ROC analysis, AUC was 82.3% (95% CI 66.9-97.8). In 4 BCM samples detected euploidy, while TE biopsy showed embryo monosomes, possibly due to mosaicism. 7 samples were excluded due to low quality cfDNA. Of the 33 samples, 7 were male (XY), according to both TE biopsy and BCM analysis, a fact that confirms the safety of the method, as it shows no contamination by maternal DNA.

Limitations, reasons for caution: The study is limited by the small sample size. To become the niPGT reliable, several steps must be optimized: DNA collection methods, DNA amplification and downstream techniques for analysis. Also, the analysis of discarded whole blastocysts as a gold standard control may determine the method's reliability.

Wider implications of the findings: Non-invasive Preimplantation Genetic Testing (niPGT), is a promishing alternative that may give an accurate and reliable option of detecting euploid human embryos, also dealing with the problem of mosaicism in trophodectoderm biopsies. Further technical refinement is needed to perfect niPGT, so that it can be used in routine clinical practice.

Trial registration number: N/A

P-528 rDNA methylation of human oocytes of women undergoing intracytoplasmic sperm injection (ICSI) increases with maternal age

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Study question: Is there a correlation between the age of women undergoing ICSI and methylation pattern of rDNA core promoter and upstream control element in immature human oocytes?

Summary answer: Methylation levels of the upstream control element and the rDNA core promoter in immature human oocytes increase with age of women undergoing ICSI.

What is known already: Methylation of ribosomal DNA (rDNA) in germ cells regulates temporary and spatially highly coordinated nucleolar activity, cellular metabolism, and thus developmental potential of the early embryo. Alterations of methylation pattern may therefore cause dysregulation of genes and signal cascades resulting in limited fertility. It has been shown that the methylation of sperm rDNA increases with the donor's age. The positive correlation between sperm rDNA methylation and age has been conserved among mammals during evolution including humans and mice. In contrast to sperm, little is known about the methylome of human oocytes and its role in human reproduction.

Study design, size, duration: Consecutive women undergoing ICSI because of male subfertility were included. Patients with endometriosis, polycystic ovary syndrome, ovarian, uterine or breast cancer, as well as patients with an anti-Mullerian hormone level <1ng/ml were excluded. Immature oocytes (germinal vesicle; GV) collected during oocyte pick-up at the Fertility Centre Dortmund between 2018 and 2020 were examined.

Participants/materials, setting, methods: Cumulus-free GV oocytes which were not usable for ICSI were rinsed in phosphate buffer and stored at -20°C until further investigation. Multiplex-PCR followed by singleplex-PCRs were carried out on the rDNA core promoter and upstream control element. Methylation levels were quantified by bisulphite pyrosequencing. Two oppositely imprinted genes (h*PEG3* and h*GTL2*) were used as controls to ensure correct amplification and bisulphite conversion. Spearman's-rank-order-correlation and Mann-Whitney-U-Test were used for statistical analysis.

Main results and the role of chance: For each GV oocyte, nine different Cytosine-phosphate-Guanine dinucleotides (CpGs) were quantified by bisulphite pyrosequencing for the rDNA core promoter and 26 different CpGs for the upstream control element (UCE). 120 human single oocytes from 60 women were analyzed. Connected statistical analysis was used if one patient had more than one oocyte. The age of the included women ranged from 26 to 40 years (mean±SD 33.5±3.2). Only oocytes which showed a correct methylation pattern for at least one imprinting control gene (h*PEG3* and h*GTL2*) were considered for analysis. Mean methylation level ranged from 2-31% (mean±SD 8.7±5.5) of

the analyzed CpGs for the rDNA core promoter and from 3-36% (mean±SD 11.4±7.1) CpGs for UCE. Spearman's correlation analysis revealed that the methylation levels of the human oocyte rDNA core promoter and rDNA UCE significantly increased with the age of the donor ($p<0.05$). Correlation coefficient for rDNA core promoter was $r=0.22$ and for upstream control element $r=0.21$. It is also interesting to note that different oocytes from the same donors can display enormous methylation variation. Regarding clinical parameters, no correlation was observed between the methylation pattern of the rDNA core promoter or UCE and the body mass index or smoking status, respectively.

Limitations, reasons for caution: Limitations of this study include difficulties in extrapolating the findings to the general population, because no data of women not undergoing ICSI are available. Only GV-oocytes were analyzed. Additional research is needed to clarify the effect of different methylation pattern with increasing female age and its role in human reproduction.

Wider implications of the findings: We propose that the increase of rDNA methylation in male and female germ cells with advanced age directly or indirectly influences the regulation of nucleolar activity, cellular metabolism, and thus the developmental potential of the early embryo. This age-dependent epigenetic effect may result in decreased human fertility.

Trial registration number: NCT03565107

P-529 Whole-genome methylation analysis of testicular germ cells from cryptozoospermic men points to recurrent and functionally relevant DNA methylation changes

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Study question: Do DNA methylation changes occur in testicular germ cells (TGCs) from patients with impaired spermatogenesis?

Summary answer: TGCs from men with cryptozoospermia exhibit altered DNA methylation levels at several genomic regions, many of which are associated with genes involved in spermatogenesis.

What is known already: In the last 15 years, several studies have described DNA methylation changes in sperm of infertile men. More recently, using whole genome bisulfite sequencing (WGBS) we were able to refute these findings by demonstrating that somatic DNA contamination and genetic variation confound methylation studies in swim-up purified sperm of severely oligozoospermic men. However, it remains unknown whether altered DNA methylation plays a role during the development of the germ cells in the testes of these patients.

Study design, size, duration: For identifying DNA methylation differences associated with impaired spermatogenesis, we compared the TGC methylomes of men with cryptozoospermia (CZ) and men with obstructive azoospermia (n=4 each), who had normal spermatogenesis and served as controls (CTR). Study participants were selected among an age-matched cohort of 24 CTR and 10 CZ. The selection was based on similar composition of the TGC suspension evaluated by ploidy analysis and absence of somatic DNA.

Participants/materials, setting, methods: TGCs were isolated from biopsies after short-term cell culture. Presence of somatic DNA was evaluated by analyzing the DNA methylation levels of *H19*, *MEST*, *DDX4* and *XIST*. WGBS was performed at ~14× coverage. Bioinformatic tools were used to compare global DNA methylation levels, identify differentially methylated regions (DMRs) and functionally annotate the DMRs. Single-cell RNA sequencing (scRNA-seq) was used to associate the DNA methylation changes to gene expression.

Main results and the role of chance: We could not identify any difference in the global DNA methylation level or at imprinted regions between CZ and CTR samples. However, using stringent filters to identify group-specific methylation differences, we detected 271 DMRs, 238 of which were hypermethylated in CZ (binominal test, $p<2.2\times10^{-16}$). The DMRs are associated with 132 genes, 61 of which are known to be differentially expressed at various stages of spermatogenesis according to scRNA-seq studies. Almost all of the DMRs associated with the 61 genes are hypermethylated in CZ (63/67, $p=1.107\times10^{-14}$). As assessed by scRNA-seq, 13 DMR-associated genes, which were mainly expressed during meiosis and spermiogenesis, show a significantly different pattern of expression in CZ patients. In four of these genes, the promoter was hypermethylated in CZ men, which correlates with a lower expression level in these patients. In the other nine genes, most of which downregulated in CZ, germ cell-specific enhancers may be affected.

Limitations, reasons for caution: The small sample size constitutes a limitation of this study. Furthermore, even though the cellular composition of samples was similar by ploidy analysis, we cannot rule out that the observed DNA methylation changes might be due to differences in the relative proportion of different germ cell types.

Wider implications of the findings: Impaired spermatogenesis is associated with DNA methylation changes in testicular germ cells at functionally relevant regions of the genome, which points to an important role of DNA methylation in normal spermatogenesis. The DNA methylation changes may contribute to premature abortion of spermatogenesis and therefore not appear in mature sperm.

Trial registration number: N/A

P-530 The use of wide thresholds for detecting intermediate chromosomal CNV up to 80% doesn't improve PGT-A ability to discriminate true mosaic from uniformly aneuploid embryos

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Study question: What is the effect of varying diagnostic thresholds on the accuracy of Next Generation Sequencing (NGS)-based preimplantation genetic testing for aneuploidies (PGT-A)?

Summary answer: When single trophectoderm biopsies are tested, the employment of 80% upper threshold increases mosaic calls and false negative aneuploidy results compared to more stringent thresholds.

What is known already: Trophectoderm (TE) biopsy coupled with NGS-based PGT-A technologies are able to accurately predict Inner Cell Mass' (ICM) constitution when uniform whole chromosome aneuploidies are considered. However, minor technical and biological inconsistencies in NGS procedures and biopsy specimens can result in subtle variability in analytical results. In this context, the stringency of thresholds employed for diagnostic calls can lead to incorrect classification of uniformly aneuploid embryos into the mosaic category, ultimately affecting PGT-A accuracy. In this study, we evaluated the diagnostic predictivity of different aneuploidy classification criteria by employing blinded analysis of chromosome copy number values (CNV) in multifocal blastocyst biopsies.

Study design, size, duration: The accuracy of different aneuploidy diagnostic cut-offs was assessed comparing chromosomal CNV in intra-blastocysts multifocal biopsies. Enrolled embryos were donated for research between June and September 2020. The Institutional Review Board at the Near East University approved the study (project: YDU/2019/70-849). Embryos diagnosed with uniform chromosomal alterations (single or multiple) in their clinical TE biopsy (n=27) were disaggregated into 5 portions: the ICM and 4 TE biopsies. Overall, 135 specimens were collected and analysed.

Participants/materials, setting, methods: Twenty-seven donated blastocysts were warmed and disaggregated in TE biopsies and ICM (n=135 biopsies). PGT-A analysis was performed using Ion ReproSeq PGS kit and Ion S5 sequencer

(ThermoFisher). Sequencing data were blindly analysed with Ion-Reporter software. Intra-blastocyst comparison of raw NGS data was performed employing different thresholds commonly used for aneuploidy classification. CNV for each chromosome were reported as aneuploid according to 70% or 80% thresholds. Categorical variables were compared using Fisher's exact test.

Main results and the role of chance: In this study, a total of 50 aneuploid patterns in 27 disaggregated embryos were explored. Single TE biopsy results were considered as true positive when they displayed the same alteration detected in the ICM at levels above the 70% or 80% thresholds. Alternatively, alterations detected in the euploid or mosaic range were considered as false negative aneuploidy results. When the 70% threshold was applied, aneuploidy findings were confirmed in 94.5% of TE biopsies analyzed (n=189/200; 95%CI=90.37-37.22), while 5.5% showed a mosaic profile (50-70%) but uniformly abnormal ICM. Positive (PPV) and negative predictive value (NPV) per chromosome were 100.0% (n=189/189; 95%CI=98.07-100.00) and 99.5% (n=2192/2203; 95%CI=99.11-99.75) respectively. When the upper cut-off was experimentally placed at 80% of abnormal cells, a significant decrease (p-value=0.0097) in the percentage of confirmed aneuploid calls was observed (86.5%; n=173/200; 95%CI=80.97-90.91), resulting in mosaicism overcalling, especially in the high range (50-80%). Less stringent thresholds led to extremely high PPV (100.0%; n=173/173; 95%CI=97.89-100.00), while NPV decreased to 98.8% (n=2192/2219; 95%CI=98.30-99.23). Furthermore, no additional true mosaic patterns were identified with the use of wide range thresholds for aneuploidy classification.

Limitations, reasons for caution: This approach involved the analysis of aneuploidy CNV thresholds at the embryo level and lacked from genotyping-based confirmation analysis. Moreover, aneuploid embryos with known meiotic partial deletion/duplication were not included.

Wider implications of the findings: The use of wide thresholds for detecting intermediate chromosomal CNV up to 80% doesn't improve PGT-A ability to discriminate true mosaic from uniformly aneuploid embryos, lowering overall diagnostic accuracy. Hence, a proportion of the embryos diagnosed as mosaic using wide calling thresholds may actually be uniformly aneuploid and inadvertently transferred.

Trial registration number: N/A

P-531 hCFTR p.G970D mutation causes Sertoli Cell-only Syndrome (SCOS) and Congenital bilateral absence of the vas deferens (CBAVD)

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Study question: Whether CFTR is a pathogenic gene for azoospermia?

Summary answer: CFTR p.G970D affects spermatogenesis and leads to male infertility by affecting the proliferation and survival of Germ Cell.

What is known already: Male infertility is a multifactorial and heterogeneous pathological condition affecting 7% of the general male population. However, up to now, only a relatively low number of genic factors have a clear relationship with spermatogenesis. Although, increased frequency of CFTR mutations or impaired CFTR expression in men with non-obstructive azoospermia or oligospermia as compared to the fertile men has been reported, but there is no direct evidence CFTR mutations cause azoospermia. Compared to F508Del mutations in Caucasians, p.G970D mutation is the most frequent CFTR mutation identified in Chinese CF patients. However, p.G970D has not been reported involved with male infertility.

Study design, size, duration: In this study, began in an infertile man suffering CBAVD and SCOS with no CF-like phenotype related symptoms up to now. By identifying the patient with CFTR p.G970D mutation, we further verified the function of the mutation in spermatogenesis in spermatogonia cell lines. Control testicular tissue sample was obtained from fertility man donors.

Participants/materials, setting, methods: WES was performed for probands and relatives and the mutation was confirmed by Sanger sequencing. Hematoxylin-eosin (HE) staining and immune fluorescence (IF) was performed on seminiferous tubules from the patient and control to characterize the structural anomalies present in the patient. GC2 mCFTRG965D cells was knocked in by the CRISPR/Cas9 gene editing system. The effects of mutations on the growth and proliferation of GC2 cells were detected by CCK8, IF, WB, BCECF staining and RT-PCR.

Main results and the role of chance: First, we identified the CBAVD and SCOS patient with homozygous missense mutations p.G970D in the CFTR gene, and his mutation inherited from both parents. The patient has normal general parameters and fertility parameters except for smaller testes, lower semen volume and pH. His testicular histopathology and co-location of CFTR and DDX4 which is the marker of spermatogonia likewise showed SCOS. Second. given that the amino acid sequence is conserved and the same expression and localization patterns of CFTR between human and mouse, we generated mouse derived cell lines model (mCFTRG965D) that carried a homozygous mutation equivalent to the *CFTR* variant in patients, using CRISPR/Cas9-mediated genome editing. mCFTRG965D affects the proliferation of Germ Cell, but has less effect on Sertoli cells, which is similar to the SCOS patient's phenotype. Third, lower mature CFTR were observed in the GC2 mCFTRG965D groups cells compared to those in wild type groups, and CFTR protein is not evident in the GC2 mCFTRG965D groups' cell membrane, which demonstrated the mutation affecting the anchoring of CFTR to the cell membrane. What's more, the missense mutation will affect the function of CFTR in regulating pH, thus affecting cell homeostasis.

Limitations, reasons for caution: The low number of biological samples, we need more patients to confirm this mutation and azoospermia. We only validated at the cellular level, not in an animal model. It is noteworthy that, the CFTRF508del mice are fertility.

Wider implications of the findings: Our study reveals that CFTR has a broader indication than just the absence of the vas deferens. We recommend to take further understanding of CFTR playing important role in spermatogenesis by affecting germ cell survival not just regulating cell volume during spermiogenesis.

Trial registration number: not applicable

P-532 Embryo quality needs to be considered as a main criterion when selecting mosaic embryos for transfer

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Study question: Should we consider embryo quality as one of the most important criteria to follow when transferring a mosaic embryo?

Summary answer: Embryo quality is an implantation biomarker both for euploid and mosaic embryos, and also a determinant for selecting the most eligible mosaic for transfer.

What is known already: Several studies show the benefit of transferring mosaic embryos when there are no euploid embryos to transfer, and they still result in ongoing pregnancies and what is more important is that they result in healthy babies.

Studies and guidelines suggest prioritizing mosaic embryos based on maternal age, chromosomes impacted, percentage of aneuploidy, number of chromosomes involved, type of mosaic (simple vs complex, segmental vs complete, monosomy vs trisomy) but embryo quality is never part of these criteria.

Studies claim that mosaic implantation rate is lower than euploid embryos, but they never show if both populations are comparable in terms of quality.

Study design, size, duration: This is a retrospective observational study performed in a private centre between February 2018 and January 2020. The study includes the data analysis of 96 euploid blastocysts and 14 low risk mosaic blastocysts (defining low risk regarding chromosome syndromes and less than 50% level mosaicism). All transferred in single embryo transfer (SET) to 105 patients after PGT-A (mean maternal age 38,9 years).

The SET factor enables us to track the implantation outcome of all embryos.

Participants/materials, setting, methods: PGT-A with NGS technology was offered to patients of advanced maternal age and/or with repeated IVF failures. Trophectoderm biopsies were performed on day 5 and/or day 6 embryos, with laser assistance. Blastocyst morphology was scored in 3 groups: A: excellent (AA, AB, BA), B: good (BB), C: average and poor-quality embryos (BC, CB, CC). (Gardner-Schoolcraft classification)

Low risk mosaic embryo transfer was offered to patients with no euploid embryos to transfer.

Main results and the role of chance: We found no significant differences between both populations (euploid and mosaic embryos) in terms of embryo quality (Chi^2 p-value =0,0975) so we were able to compare the overall implantation of similar quality populations.

Despite euploid implantation being higher as described in most studies, no statistical differences (Chi^2 p-value = 0,4344) were found in terms of implantation rates between mosaic (57,0%) and euploid (67,6%) blastocysts during the same period. There are no differences between the mean age of both groups (39,7 vs 38,8 years, respectively).

The implantation rates for euploid blastocysts were 79,5% (n=39), 62,7% (n=51) and 33,3% (n=6) in the A, B and C blastocyst quality groups, respectively, showing significative differences among the three groups.

The implantation rates of low-risk mosaic blastocysts were 100% (n=3), 62,5% (n=8) and 0,0% (n=3) in the A, B and C blastocyst morphology groups, respectively, showing also still significant differences among the three groups despite the small population. (Chi^2 p-values according to implantation: Euploid =0,0434; Mosaic=0,0419)

We have also compared the three quality categories between both populations showing no significative differences (Chi^2 p-values according to quality: A=0,4344; B=0,9894; C=0,2568), concluding that same quality embryos behave the same way despite being euploid or mosaic.

Limitations, reasons for caution: The study is limited by its retrospective nature and the low number of mosaic embryos transferred as they are the last option for transfer. Additionally, it is common to transfer more than one mosaic embryo to increase the chances of pregnancy, therefore losing implantation track.

Wider implications of the findings: Embryo quality has always been a strong biomarker predictable for implantation and this is also true for mosaic embryos as well. It is a simple concept, but we cannot compare implantation potential of euploid embryos with mosaic embryos without describing both populations in terms of quality.

Trial registration number: not applicable

P-533 Effects of sex chromosomal complement, XX, XO, or XY, on the transcriptome and development of mouse oocytes during follicular growth

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Study question: How does the sex chromosome complement affect the transcriptome and development of oocytes during follicular growth in the mouse ovary?

Summary answer: Highly expressed X-linked genes adjust their transcript levels according to the X dosage. Y-linked genes affect the transcript levels of some X-linked and autosomal genes.

What is known already: Female mice carrying XO and XY chromosomes on the C57BL/6J (B6) genetic background are healthy but encounter subfertility and infertility, respectively. Our previous results have shown that the XY oocyte is defective in its cytoplasm; its replacement with that of an XX oocyte at the GV stage allows for production of healthy offspring after fertilization. Since transcription is shut down in the oocyte by the end of growth phase, the mRNAs and proteins necessary for meiotic progression and early embryonic development are accumulated during follicular growth.

Study design, size, duration: 30 oocytes of 50-59 µm diameter were pooled for each genotype in biological triplicate and subjected to RNA-Sequencing. Total RNA extracted from 10-30 pooled oocytes of each size range and genotype in biological triplicate were subjected to qRT-PCR. All experiments were performed between 2019-2021.

Participants/materials, setting, methods: XY and XO females were generated by cross between B6 females with B6.YTIR and B6.XPafY males, respectively. Oocytes in the growth phase were collected at 8-18 days postpartum (dpp), whereas fully-grown oocytes were collected at 27-29 dpp after injection with equine chorionic gonadotropin. Oocytes of 50-59 µm diameter were subjected to RNA-Sequencing using a version of SmartSeq2, followed by DEG analyses. Transcript levels in the oocytes of various diameters were determined by qRT-PCR.

Main results and the role of chance: Chromatin configuration, mitochondrial distribution, and de novo transcription were largely comparable among the XX, XO, and XY oocytes smaller than 60 µm. Three way comparisons of RNA-Seq data in the oocytes of 50-59 µm revealed; (1) 13.8% of X-linked DEGs showed the transcript levels in correspond to the X chromosome dosage; (2) 9 genes on the Y short arm and 2 genes near the distal end of the Y long arm were highly expressed in XY oocytes; (3) transcript levels of X- or autosomal homologs were affected by the XY complement compared to XX and XO oocytes; and (4) 54 and 39 X-linked and autosomal genes show higher and lower transcript levels, respectively, in XY oocytes compared to XX and XO oocytes. The results of qRT-PCR of selected genes revealed distinct dynamic changes in transcript levels in the oocyte during follicular growth. Data of RNA-Seq were statistically analyzed using R Bioconductor limma package for differentially expressed genes having Benjamini-Hochberg adjusted *P* values lower than 0.01 and log2 fold change higher than 1. All data of qRT-PCR were statistically analyzed by one-way ANOVA followed by Tukey's honestly significant difference (HSD) test.

Limitations, reasons for caution: In humans, most XO females die in utero and those who reach the term suffer from congenital abnormalities and infertility (Turner's syndrome). However, the severer phenotype can be attributed to somatic cells with a greater number of genes that escape from X chromosome inactivation in humans than mice.

Wider implications of the findings: XO and XY mice provide animal models for investigating the consequence of X haplodeficiency in the female germline, independent of somatic defects. Furthermore, XY female mice provide a unique opportunity for examining whether and how Y-linked genes are transcribed outside the male germline.

Trial registration number: not applicable

P-534 A novel heterozygous mutation in the luteinizing hormone/choriogonadotropin receptor (LHCGR) gene in a patient with 'genuine' empty follicle syndrome

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Study question: Whether empty follicle syndrome (EFS) in a patient has a genetic basis.

Summary answer: Our findings would expand the mutational spectrum of *LHCGR*, in patients with GEFS

What is known already: The LHCGR gene (OMIM #52790) is located on chromosome 2p21 has 11 exons The LHCGRs present in gonadal cells; granulosa, theca and luteal cells in women and Leydig cells in menandplays a critical role in male sexual differentiation, female ovarian development and fertility (folliculogenesis, ovulation, corpus luteum formation and progesterone secretion)

Inactivating mutations in males can lead to Leydig cell hypoplasia, which causes disorders in sexual development (MIM #238 320). Phenotype of women is less severe and variable and has no effect on the secondary sex characteristics, but it could cause amenorrhoea and infertility Study design, size, duration: In the context of clinical genetics, Next Generation Sequencing libraries were prepared in line with the manufacturer's orders using QIASeq™ Targeted DNA Custom Panel (Qiagen) targeting exons and 20 bp exon-intron boundaries of selected genes (AR, BMP15, CATSPER1, CFTR, CYP21A2, FSHB, FSHR, HESX1, LHB, LHCGR, NR5A1, POU1F1, SRY, ZP1). The variant detected in NGS analysis was further confirmed using Sanger sequencing (MiSeq, Illumina, San Diego, CA).

Participants/materials, setting, methods: A 27 years old Turkish women with 6 year history of primary unexplained infertility underwent controlled ovarian hyperstimulation and IVF with an antagonist protocole in Ankara City Hospital. Although normal follicular development, E2 levels, and bioavailable β-hCG plasma levels, no oocytes or cumulus-corona complexes were retrieved by follicular aspiration.

Main results and the role of chance: A novel heterozygous mutation on Exon 5 of LHCGR (NM_000233.4):c.453C>G (p.Phe151Leu). This variant has not been reported in GnomAD database and is a novel variant as per controlled from ClinVar and HGMD mutation databases

Also coagulation tests were done Patient was heterozygote for the prothrombin mutation (FXIII) and homozygote for MTHFR C677T mutation. The patient has normal pubertal development and female karyotype (46,XX). Gonadotropin and E2 levels were normal, nor any history of anosmia, primary amenorrhoea, polycystic ovaries, hyperandrogenism, systemic disorder, or neurologic defect.

According to ACMG 2015 and HGMD detected variant was classified as unknown clinical significance (VUS) variant In 22nd World Congress COGI in 2015 we have presented another recurrent GEFS case with compound heterozygous frameshift mutations in exon 5 and 11 of LHCGR*gene*(c.1764_1765 insT) and (c.430G>T, p.V144F) who developed premature ovarian failure at the end The different types of LHCGR mutations would lead to the different functional effects and clinical phenotypes in different ages; primary amenorrhea with high levels of LH, poor ovarian response (Poesidon group1), GEFS and secondary amenorrhea (premature ovarian failure) GEFS may be a gradual biological occurrence related to ovarian ageing. Long term follow up is important, since some clinical manifestations appear later in life.

Limitations, reasons for caution: LH resistance in females were always found due to their effected brothers and only 10 GEFS cases of 46, XX females with LHCGR gene defect reported in literature.

Patient was heterozygous for indicated mutation; therefore segregation study should be done in family members and in vitro studies should be performed.

Wider implications of the findings: Screening for mutations in the LHCGR gene in Poseidon group 1 and in patients GEFS especially the recurrent ones will provide valuable information and time for clinical management, corresponding treatment and give the opportunity of wise and cost effective counseling of patients about their future reproductive choices.

Trial registration number: it is a case report

P-535 Machine learning classification algorithms can predict the ploidy status on day 5 and 6 with a 79% accuracy using evolutive morphological parameters and patient age

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Study question: Can Machine Learning predict ploidy status from embryo evolutive morphological parameters in a non intrusive way?

Summary answer: Reporting cumulative embryo scoring from early development days and feed it to machine learning algorithms can help better predict the ploidy status of embryos

What is known already: Wiemer and Kelley showed morphological parameters and development rates were important parameters to consider during the embryo transfer process. Minasi et al. showed morphology assessment at blastocyst stage was correlated with ploidy status. Chavez-Badiola et al. showed a deep learning algorithm could predict the embryos ploidy with an accuracy of 70% and with positive predictive value of 0.79 using labeled blastocysts images.

Study design, size, duration: Study was a retrospective cohort analysis from 2019 to 2020 of 2520 biopsied embryos (669 cycles) cultured at POMA fertility clinic. Observations included all autologous embryos undergoing PGTA on day 5 or 6 with known PGTA status. Embryos from donors and with undefined PGTA results were excluded from the study. The embryos were classified as either euploid (n=1528) or displaying a chromosomal anomaly (n=992)

Participants/materials, setting, methods: Input of the machine learning model included patient age and 19 morphological parameters collected on days 1, 2, 3, 5 and 6 (symmetry, number of cells, blastocyst status, fragmentation, ICM and troph grades). An xgboost algorithm was trained on 80% of the data (n= 2016) and tested on 20% of blind data (n=504).

Main results and the role of chance: Xgboost machine learning algorithm managed to predict ploidy status on the blind dataset with an accuracy of 79%, significantly higher than random chance (AUC= 0.71) and a positive predictive value of 87%.

Blastocyst stage parameters that are usually monitored to assess embryo quality (ICM, troph and blastocyst status on days 5 and 6) ranked high in the predictive power of the machine learning algorithm. It appeared that despite moderate to small individual correlation with the ploidy status, including parameters on day 1 (including number of PNs and number of cells on day1 PM check) to day 3 increased the performance of the classification algorithm from 70% accuracy to 79% accuracy. Machine learning algorithms are able to exploit evolution of morphological parameters during the development to improve the prediction.

Limitations, reasons for caution: Dataset was derived from one single clinic with manual annotations. Results should be validated on more clinics and inter-operator variation in morphological parameters annotation should be assessed to confirm robustness of the model and increase its predictive power.

Wider implications of the findings: Study shows the potential of detecting ploidy status in a non intrusive way and participating to embryo selection. Study confirms the importance of annotating morphological parameters of embryos in the early days of development.

Trial registration number: not applicable

P-536 Validation of a Next Generation Sequencing (NGS) workflow integrating simultaneous analysis of ploidy, microdeletions and de novo monogenic diseases for expanded preimplantation genetic testing (PGT)

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Study question: Can major *de novo* genetic and chromosomal abnormalities (i.e., ploidy, microdeletions) be effectively tested on a single embryo biopsy specimen using an integrated NGS approach?

Summary answer: The integrated NGS workflow provided high accuracy for multilevel chromosome and genetic abnormalities analysis based on single biopsies expanding PGT informativity to *de novo* conditions.

What is known already: Current NGS-based methodologies employed in PGT for aneuploidy (PGT-A) do not detect embryo ploidy level nor frequent pathogenic *de novo* microdeletions below resolution limits. Moreover, despite their considerable incidence and adverse pregnancy outcomes, *de novo* mutations causing severe dominant monogenic fetal structural defects (FSD) are not investigated during PGT. The development of a single biopsy specimen-based PGT-A sequencing strategy that integrates ploidy and *de novo* microdeletions/mutations assessment would significantly widen PGT-A diagnostic scope and technical capabilities. This comprehensive approach would provide additional valuable genetic information of unquestionable clinical utility to further refine embryo selection process among those showing euploid profiles.

Study design, size, duration: Chromosomal conditions were validated using 24 embryo rebiopsies and 5 cell lines with both known ploidy level and known microdeletions (-4p; -8q; -1p; -22q; -5p; -15q; -11q). Genotyping for monogenic conditions was validated using 5 genomic DNA samples (33pg/µl) carrying known pathogenic Single Nucleotide Variants (SNVs) in COL1A1, SOS1, PTPN11, TSC2 and FGFR2 genes. To assess technical performance across identified SNPs, genotyping accuracy was evaluated on 17 samples from 5 embryos and 2 cell lines.

Participants/materials, setting, methods: Thirty-two *de novo* dominant monogenic conditions with FSD and strong gene-disease relationship were tested using a multiplex PCR panel with sequencing for the genes' whole coding region. Eight common microdeletions (<10Mb) syndromes (Wolf-Hirshorn, Langer-Geidion, 1p36 deletion, De George, Cri-du-Chat, Prader-Willy/Angelman, Jacobsen) were tested using B-allelic frequency (BAF) of 356 highly polymorphic

Single Nucleotide Polymorphisms (SNPs). These SNPs were also used for ploidy assessment. Library preparation and sequencing were performed on the IonTorrent S5 (ThermoFisher).

Main results and the role of chance: Blinded NGS data analysis confirmed the ploidy status in all (19) samples with known constitution (8 diploids, 7 polyploids, 4 haploids). Specifically, the proportion of heterozygote calls (BAF 40%-60%) was 60.9% (95%CI:47.6-72.8) for diploid samples and <1% for haploid samples(P<0.001). All polyploid samples showed a typical splitting of BAF among 3 experimental ranges (20-40%,40%-60%,60-80%): 34.1%,18.2% and 47.7%, respectively. For microdeletions, all interstitial SNPs genotyped showed a loss of heterozygosity (LOH) as expected. The analysis of positive controls consisting of 20 blastocyst rebiopsies and 3 cell lines (-4p: n=3; -8q: n=4; -1p: n=5; -22q: n=3; -5p: n=2; -15q: n=4; -11q: n=2), allowed to accurately characterize 6 out of the 7 microdeletions (18/23 samples). In particular, all interstitial SNPs genotyped showed a LOH, while diploid controls showed an overall heterozygosity of 30.9% (average number of hetSNP x deletion=9/28). Only the very small telomeric 1p36 region failed to properly amplify. For monogenic conditions, sequencing analysis of 5 positive gDNA controls confirmed the presence of 4 known SNVs, whilst only 1 did not achieve the minimum coverage for variant calling. Moreover, 4 additional *de novo* SNVs detected by sequencing analysis in the gene panel on 8 blastocyst rebiopsies were all confirmed by qPCR/Taqman assays.

Limitations, reasons for caution: Positive controls were not available for all genes and microdeletions included in the panel. Moreover, inefficient amplification has affected some target regions and further optimization will be required. However, analytical performance on technical and biological replicates were highly promising for the tested conditions both cell lines and trophectoderm biopsies.

Wider implications of the findings: This study demonstrates that the integration of genotyping and chromosomal analyses can be efficiently achieved in the same NGS workflow. This approach can be employed to expand PGT diagnostic scope to conditions undetectable in parents due to their *de novo* onset, or that are below the standard PGT-A resolution.

Trial registration number: N/A

P-537 What is the attitude of gamete donors towards expanded genetic testing

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Study question: What is the opinion of gamete donors on extended carrier screening in Denmark and in US?

Summary answer: This study showed that the overwhelming majority of the donors were very positive towards genetic testing in general and the expanded carrier screening.

What is known already: There is a lack of empirical studies on the experiences of and attitudes of donors towards expanded carrier screening (ECS) (Amor et al. 2018). Oocyte donors in a Spanish clinic were surprised by the information on testing and the possibility of being carriers (Abuli et al., 2016). After adequate genetic counselling before and after the test, the results of testing did not seem to have a meaningful emotional or psychological impact on the donors.

Study design, size, duration: A questionnaire was emailed to all active sperm donors in a sperm bank in Denmark and in a sperm bank in US.

Participants/materials, setting, methods: A total of 393 donors received the email of which 233 donors completed the questionnaire, 196 in Denmark (response rate of 60.7%) and 37 in the United States (response rate of 52.9%).

Main results and the role of chance: We systematically compared the US and DK donors and ID-release versus non-ID-release donors for all characteristics and items. ID-release donors with a partner significantly more informed their partner about their donor status than non-ID-release donors (90.5% vs. 72.4%, p 0.027). ID-release donors significantly more intended to tell their own children (57.5% vs. 21.2%, p 0.001). ID-release donors significantly more thought about their potential donor children (56.2% vs. 35.0%; p 0.002) and significantly more likely to want information on whether a pregnancy occurred from their donation (70.6% vs. 55.0%, p 0.017). In addition, they also significantly more wished to obtain information about the children conceived with their sperm: the number of children (64.0% vs. 50.0%, p 0.048), their gender (40% vs. 26.2%, p 0.042), and their health (59.3% vs. 42.5%, p 0.018). The ID-release donors were much less likely than the other type not to want any information (19.3% vs. 32.5%, p 0.034). This general attitude is extended to the wish to be informed if a child with a hereditary disease is born after using their sperm. The ID-release donors were more likely to desire contact if their genetic child was diagnosed with a genetic disease (73.9% vs. 55.0%, p 0.003).

Limitations, reasons for caution: The main element that makes the study unique (i.e., the choice between ID-release and non-ID-release) also renders the generalization of the findings more difficult. Wider implications of the findings: A better understanding of the attitude among gamete doners will possibly guide future legislation and ensure the safety and welfare of the donors too.

Trial registration number: none

P-538 Potential involvement of testicular extracellular vesicles in the paternal epigenetic inheritance of undesirable metabolic traits

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Study question: Does high-fat diet alter the cargoes of testicular extracellular vesicles (tEVs) and thus modulate the sperm epigenome?

Summary answer: The properties and small RNA cargoes of tEVs and the sperm epigenome were significantly altered in mice fed with a high-fat diet.

What is known already: High-fat diet is known to alter spermatogenesis and sperm quality. Recent studies showed that the undesirable metabolic traits can be inherited to the next generation via paternal epigenetic inheritance. Hitherto, it has been shown that the extracellular vesicle, an important intercellular communication pathway, secreted by the epididymis conveys small RNA cargoes to sperms and mediate paternal epigenetic inheritance of metabolic traits. Surprisingly, although the sperm are first being produced in the testes, the potential contributions of testicular EVs (tEVs) in the sperm epigenome remain unexplored.

Study design, size, duration: It is a proof-of-concept study using mice as an experimental model. Thirty mice were raised for nine months, high-fat diet (HFD) and chow diet (SD) were treated on each half of the subject group starting from the sixth week until they were euthanized. Participants/materials, setting, methods: The study is conducted under laboratory settings. Sperm and tEVs were obtained from mice fed with HFD or SD. The uptake of tEVs by sperm was monitored by flow cytometry analysis using fluorescence-labelled tEVs. Physical properties of testicular EVs were examined by the transmission electron microscope. The small RNA cargoes were investigated by small RNA sequencing. The sperm epigenome was examined by real-time-qPCR.

Main results and the role of chance: Our results showed that sperm efficiently took up the tEVs in a dose-dependent manner, without compromising the sperm motility. Size of tEVs in HFD-fed mice (320.5 ± 99.83 nm) was significantly greater than that of SD-fed mice(251.9 ± 81.01 nm). RNA sequencing revealed a decrease in the percentage of miRNA in HFD tEVs. Eight miRNAs were differentially expressed in HFD tEVs.

Among them, real-time PCR results confirmed that miR-34b and c levels were significantly up-regulated in HFD tEVs, with a log2[Fold-change] of 0.46613 and 0.42935 respectively. Unexpectedly, the levels of both miR-34b and c were similar in HFD and SD epididymis, and were both down-regulated by about 2-fold in matured sperm of HFD-fed mice. To investigate the cause of discrepancy, we carried out flow cytometry analysis to measure the absorption efficiency of tEVs, which revealed a notable decrease in absorption efficiency of HFD tEVs (70.235 ± 4.864 %) by sperms compared to that of SD tEVs (79.350 ± 4.012 %).

Limitations, reasons for caution: Cauda sperm was used in the profiling of sperm epigenome where the contributions from the epididymosomes have not been compared. The study was conducted using mice models such that discrepancy may occur when applying to humans.

Wider implications of the findings: We revealed the alteration of tEVs in HFD-fed mice which may underlie the perturbation of spermatogenesis in HFD

condition. We demonstrated the efficient uptake of tEVs by sperm which may be developed as a tool for the engineering of the sperm epigenome.
Trial registration number: not applicable

P-539 The use of expanded carrier screening of gamete donors

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Study question: What are the sperm and egg donor rejection rates after expanded carrier screening (ECS)?
Summary answer: Using an ECS panel looking at 46/47 genes, 17.6% of donors were rejected.
What is known already: The use of ECS is becoming commonplace in assisted reproductive technology, including testing of egg and sperm donors. Most national guidelines recommend rejection of donors if they are carriers of a genetic disease. If the use of ECS increases, there will be a decline in the number of donors available.
Study design, size, duration: A review of the current preconception ECS panels available to donors was carried out through an online search. The genetic testing results of donors from Cryos International were analysed to determine how many were rejected on the basis of the ECS.
Participants/materials, setting, methods: Data on gamete donors and their carrier status was provided by Cryos International, who screen donors using their own bespoke ECS panel. The ECS panels identified through the review were compared to the Cryos International panel and data.
Main results and the role of chance: A total of 16 companies and 42 associated ECS panels were reviewed. There were a total of 2673 unique disorders covered by the panels examined, with a mean of 329 disorders screened. None of these disorders were common to all panels. Cryos International screen 46 disorders in males and 47 in females. From 883 candidate donors, 17.6% (155/883) were rejected based on their ECS result. Carriers of alpha-thalassaemia represented the largest proportion of those rejected (19.4%, 30/155), then spinal muscular atrophy (15.5%, 24/155) and cystic fibrosis (14.8%, 23/155).
Limitations, reasons for caution: Panel information was found on company web sites and may not have been accurate.
Wider implications of the findings: This study highlights the need for consistent EU regulations and guidelines which allow genetic matching of gamete donors to recipients, preventing the need to reject donors who are known carriers. A larger ECS panel would be most beneficial, however, this would not be viable without matching of donors and recipients.
Trial registration number: Not applicable

P-540 A feasible diagnostic approach for the cryptic subtelomeric traslocations in early recurrent miscarriage patients by preimplantation genetic testing (PGT).

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Study question: Could cryptic subtelomeric traslocations in early recurrent miscarriage patients be diagnosed by preimplantation genetic testing?
Summary answer: PGT is a powerful tool to detect subtelomeric cryptic traslocations identifying the cause of early recurrent miscarriage and allowing subsequent genetic counselling. What is known already: Chromosome translocations are frequently associated with birth defects, spontaneous early pregnancy losses and infertility. However, submicroscopic traslocations (so-called cryptic traslocations) are too small to be detected by conventional karyotyping.. Due to balanced status, high resolution molecular techniques as arrayCGH are not able to detect it. Thus, cryptic traslocations detection is challenging. PGT is able to detect CNVs at higher resolution than routine karyotyping. Therefore, the recurrent diagnosis of CNV at embryo level could suggest a subchromosomal parental traslocation. The aim of this study is to investigate the feasibility of using PGT as an indicator of parental balanced cryptic traslocations.
Study design, size, duration: We included three couples who underwent PGT for unexplained repeated pregnancy loss (RPL) in our clinic from February 2020 to November 2020. Common established causes of RPL (uterine anomalies, antiphospholipid syndrome, immunological, hormonal and metabolic disorders) were previously rouled-out. Even couple karyotypes were normal. Twenty-three embryos from those couples were biopsied at blastocyst and analysed for CNVs detection using low coverage whole genome NGS.
Participants/materials, setting, methods: PGT by NGS was performed by Veriseq-NGS (Illumina), with previous whole genome amplification. Fluorescence in situ hybridization (FISH) using parental blood samples were performed to validate the origin of subchromosomal number variation. Commercially available subtelomeric specific probes were selected according to the CNV identified and the procedures were performed according to the manufacturer's protocols.
Main results and the role of chance: Overall, CNVs of terminal duplication and deletion that imply unbalanced traslocation derivatives were detected in the 43.5% of biopsied embryos. For couple 1, 4 out of 5 embryos (80%) carried deletion of telomeric region on chromosomes 5 and 21. Three out of 6 biopsed embyos (50%) were diagnosed with subchromosomal copy variants at telomeric region on chromosomes 6 and 16 for couple 2. In the case of couple 3, three out of 12 embryos (25%) were carriers of CNV at subtelomeric region on chromosomes 2 and 6. The size of CNVs detected ranges from 8Mb to 20Mb. Accurate diagnosis with the parental study was made by FISH. The combination of probes to detect the structural chromosome alteration were: Tel5qter-LSI21q, Tel6pter-CEP16 and Tel6pter-CEP6 for each couple respectively. The FISH studies reveal that CNVs were inherited from one parent carrying the balanced cryptic traslocation. Ultimately, the abnormal karyotype from the carrier parent were 46,XY,t(5;21)(q33.2;q21.2) for couple 1, 46,XY,t(6;16)(p22.3;q22.1) for couple 2 and 46,XY,t(2;6)(p25.1;p24.2) for couple 3. Finally, each couple performed a cryotransfer of a single normal balanced embryo. Two pregnancies are ongoing.
Limitations, reasons for caution: The main limitation of this approach is the NGS- PGT resolution. CNVs smaller than 5Mb could not be detected.
Wider implications of the findings: This study shows the value of PGT for unexplained RPL, followed by parental FISH to better characterize CNVs and identify couples in whom one partner carries a cryptic translocation. Accurate diagnosis of parental chromosome translocation can achieve with FISH only, but FISH would not be performed unless PGT showed CNVs.
Trial registration number: Not applicable

P-541 Identification of novel variants and candidate genes in women with familial idiopathic premature ovarian failure using whole-exome sequencing

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Study question: Is it possible to identify a genetic cause of familial premature ovarian failure (POF) with whole-exome sequencing (WES)?
Summary answer: Whole-exome sequencing is the most efficient strategy to identify probably pathogenic mutations in different genes in pathologies of polygenic etiology such as premature ovarian failure.
What is known already: Premature ovarian failure is the loss of ovarian function before the age of 40, and it is a common cause of infertility in women. This pathology has a heterogeneous etiology. Some chromosomal and genetic alterations have been described, and could explain approximately 20% of cases. However, in most patients the origin remains unknown. Recent studies with next-generation sequencing (NGS) have identified new variants in candidate genes related with premature ovarian insufficiency (POI) or premature ovarian failure (POF). These genes are not only involved in processes such as folliculogenesis, but also with DNA damage repair, homologous recombination, and meiosis.
Study design, size, duration: Fourteen women, from 7 families, affected by idiopathic POF were included in the study from October 2019 to September 2020. Seven POF patients were recruited when they came to our clinic to

undergo assisted reproductive treatment. In the anamnesis, it was found that they had relatives with a diagnosis of POF, who were also recruited for the study. The inclusion criteria were amenorrhea before 38 years old and analytical and ultrasound signs of ovarian failure.

Participants/materials, setting, methods: WES was performed using TrusightOne (Illumina®). Sequenced data were aligned through BWA tool and GATK algorithm was used for SNVs/InDel identification. VCF files were annotated using Variant Interpreter software. Only the variants shared by each family were extracted for analysis and these criteria were followed: (1) Exonic/splicing variants in genes related with POF or involved in biological ovarian functions (2) Variants with minor allele frequency (MAF) ≤0.05 and (3) having potentially moderate/strong functional effects.

Main results and the role of chance: Seventy-nine variants possibly related with the POF phenotype were identified in the seven families. All these variants had a minor allele frequency (MAF) ≤0.05 in the gnomAD database and 1000 genomes project. Among these candidate variants, two were nonsense, six splice region, one frameshift, two inframe deletion and 68 missense. Thirty-two of the missense variants were predicted to have deleterious effects by minimum two of the four in silico algorithms used (SIFT, PolyPhen-2, MutationTaster and PROVEAN). All variants were heterozygous, and all the families carried three or more candidate variants. Altogether, 43 probably damaging genetic variants were identified in 39 genes expressed in the ovary and related with POF/POI or linked to ovarian physiology. We have described genes that have never been associated to POF pathology, however they may be involved in key biological processes for ovarian function. Moreover, some of these genes were found in two families, for example *DDX11*, *VWF*, *PIWIL3* and *HSD3B1*. *DDX11* may function at the interface of replication-coupled DNA repair and sister chromatid cohesion. *VWF* gene is suggested to be associated with follicular atresia in previous studies. *PIWIL3* functions in development and maintenance of germline stem cells, and *HSD3B1* is implicated in ovarian steroidogenesis.

Limitations, reasons for caution: Whole-exome sequencing has some limitations: does not cover noncoding regions of the genome, it also cannot detect large rearrangements, copy-number variants (large deletions/duplications), mosaic mutations, mutations in repetitive or high GC rich regions and mutations in genes with corresponding pseudogenes or other highly homologous sequences.

Wider implications of the findings: WES has previously shown to be an efficient tool to identify genes as cause of POF, and has demonstrated the polygenic etiology. Although some studies have focused on it, and many genes are identified, this study proposes new candidate genes and variants, having potentially moderate/strong functional effects, associated with POF.

Trial registration number: Not applicable

P-542 Relationship between the single nucleotides polymorphisms in Mitochondrial Nicotinamide Adenine Dinucleotide Hydride dehydrogenase (NADH) Subunit 4 gene (MT-ND4) and male infertility

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Study question: Is there any association between male infertility and the polymorphic variants of Mitochondrial Nicotinamide Adenine Dinucleotide Hydride dehydrogenase (NADH) Subunit 4 (*MT-ND4*)?

Summary answer: Our findings suggested that male infertility was correlated to rs2853495 and rs869096886 SNPs in *MTND4*.

What is known already: The rate of mutations in the mtDNA, the powerhouse of the cell, is high due to the lack of histones and DNA repair mechanisms. Therefore, mutations that occur in the mitochondrial genome, play a major role in some human genetic disorders. 15 - 30% of male infertility are related to genetic predisposition. Sperm containing defective mitochondria cannot effectively produce ATP and more likely to produce reactive oxygen species (ROS) and free radicals, thereby causing a defect in mtDNA, make trouble energy, and deteriorate motility and fertility. Study design, size, duration: A prospective study carried out between 2018 and 2019. 112 semen samples were collected in this study.

Participants/materials, setting, methods: The present study was carried out at the department of Obstetrics and Gynecology, University of Saarland, Germany. Samples were divided into 68 subfertile and 44 fertile men. Mitochondrial DNA was extracted using a QIAamp DNA Mini Kit, after that the mtDNA was amplified by using REPLI-g Mitochondrial DNA Kit. Polymerase chain reaction (PCR) was used to amplify MT-ND4 gene. Then, samples were purified and sequenced using the Sanger method in the Microsynth Seq lab, Germany.

Main results and the role of chance: The genotypes frequencies of the study population showed a statistically significant association between rs2853495 G>A (Gly320Gly) and male infertility (P = 0.0351). Similarly, the allele frequency test showed that rs2853495 G>A (Gly320Gly) and rs869096886 A>G (Leu164Leu) were significantly associated with male infertility (adjusted OR = 2.616, 95% CI = 1.374 - 4.983, P = 0.0028; adjusted OR = 2.237, 95% CI = 1.245 - 4.017, P = 0.0073, respectively). On the other hand, no statistically significant difference was observed between the asthenozoospermia, oligozoospermia, teratozoospermia, asthenoteratozoospermia, oligoasthenoteratozoospermia, oligoteratozoospermia subgroups of subfertile males and the fertile ones.

Limitations, reasons for caution: The size number of the study samples.

Wider implications of the findings: A larger prospective study will be required to confirm these associations of mitochondrial gene polymorphisms rs2853495 and rs869096886 in *MT-ND4* and male infertility and to clarify the definite effect of the mitochondrial genetic variations on male infertility.

Trial registration number: not applicable

P-543 Inhibition of cell proliferation and extracellular matrix formation in human uterine leiomyomas by 5-aza-2'-deoxycitidine via Wnt/β-catenin pathway

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Study question: Is DNA methylation reversion through DNA methyltransferases (DNMT) inhibitors, such as 5-aza-2'-deoxycitidine, a potential therapeutic option for treatment of patients with uterine leiomyomas (UL)?

Summary answer: 5-aza-2'-deoxycitidine reduces proliferation and extracellular matrix (ECM) formation by inhibition of Wnt/β-catenin pathway on UL cells, suggesting DNMT inhibitors as an option to treat UL. What is known already: UL is a multifactorial disease with an unclear pathogenesis and inaccurate treatment. Aberrant DNA methylation have been found in UL compared to myometrium (MM) tissue, showing hypermethylation of tumor suppressor genes, which contributes to the development of this tumor. The use of DNMT inhibitors, such as 5-aza-2'-deoxycytidine (5-aza-CdR), has been suggested to treat tumors in which altered methylation pattern is related to tumor progression, as occurs in UL. Based on this, we aimed to evaluate whether DNA methylation reversion through 5-aza-CdR reduces cell proliferation and ECM formation in UL cells, being a potential option for UL medical treatment.

Study design, size, duration: Prospective study comparing UL versus MM tissue and human uterine leiomyoma primary (HULP) cells treated with/without 5-aza-CdR at 0 μM (control), 2 μM, 5 μM and 10 μM for 72 hours. UL and MM tissue were collected from women without any hormonal treatment for the last 3 months (n=16) undergoing myomectomy or hysterectomy due to symptomatic leiomyoma pathology. Participants were recruited between January 2019 and February 2020 at Hospital Universitario y Politecnico La Fe (Spain).

Participants/materials, setting, methods: Samples were collected from Caucasian premenopausal women aged 31-48 years, with a body mass index of <30 and without hormonal treatment. *DNMT1* gene expression was analysed in UL vs MM tissue by qRT-PCR and activity of DNMT was measured in UL and MM tissue and cells by ELISA**.** 5-aza-CdR effect on proliferation was assessed by CellTiter test and Western blot (WB), apoptosis and ECM analyzed by WB and Wnt/β-catenin pathway by qRT-PCR and WB. Main results and the role of chance: *DNMT1* gene expression was increased in UL compared to MM tissue (fold change

[FC]=2.49, p-value [p]=0.0295). Similarly, DNMT activity was increased in both UL compared to MM tissue and HULP cells versus MM cells (6.50 vs 3.76 OD/h/mg, p=0.026; 211.30 vs 63.67 OD/h/mg, p=0.284, respectively). After 5-aza-CdR treatment, cell viability of HULP cells was reduced in a dose dependent manner, being statistically significant at 10 μM (85.25%, p=0.0001). Accordantly, PCNA protein expression was significantly decreased at 10 μM in HULP cells (FC=0.695, p=0.034), demonstrating cell proliferation inhibition. Additionally, 5-aza-CdR inhibited ECM protein expression in HULP cells in a dose-dependent manner being statistically significant at 10 μM for COLLAGEN I (FC=0.654, p=0.023) and PAI-1 (FC=0.654, p=0.023), and at 2 μM and 10 μM for FIBRONECTIN (FC=0.812, p=0.020; FC=0.733, p=0.035; respectively). Final targets of Wnt/β-catenin pathway were decreased after 5-aza-CdR treatment, protein expression of WISP1 was significantly inhibited at 10 μM (FC=0.699, p=0.026), while expression levels of Wnt/β-catenin target genes *C-MYC* (FC=0.745, p=0.028 at 2 μM; FC=0.728, p=0.019 at 10 μM) and *MMP7* (FC=0.520, p=0.003 at 5 μM, FC=0.577, p=0.007 at 10 μM) were also significantly downregulated in HULP-treated cells vs untreated cells. Limitations, reasons for caution: This study has strict inclusion criteria to diminish epigenetic variability, thereby we should be cautious extrapolating our results to general population. Besides, this is a proof of concept with the inherent cell culture limitations. Further studies are necessary to determine 5-aza-CdR dose and adverse effects on UL *in vivo*.

Wider implications of the findings: 5-aza-CdR treatment reduces cell proliferation and ECM formation through Wnt/β-catenin pathway inhibition, suggesting that inhibition of DNA methylation could be a promising new therapeutic approach to treat UL.

Trial registration number: Not applicable

P-544 The results of embryo transfer cycles with low mosaic embryos: A case report

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Study question: What are the results of embryo transfer cycles with low mosaic embryos?

Summary answer: The clinical and ongoing pregnancy rates were 50%. The results of Amniocentesis were reported as normal karyotype for all the pregnant women.

What is known already: Many current studies, quote the rates of mosaicism in blastocyst biopsies to be higher at 20-30%. Embryonic mosaicism was found to result from mitotic errors occurring after fertilization, occasionally in the first cleavage but more commonly in the second or third cleavage. The increased reporting of mosaicism in embryos has given rise to new challenges in PGS results interpretation and patient counseling. Previously, embryos were diagnosed as either euploid or aneuploid, and in most cases only euploid embryos were considered for transfer. Now, if mosaic embryos are considered to represent a third category of results.

Study design, size, duration: Case Report for The results of 10 embryo Transfer Cycles with Low Mosaic Embryos Participants/materials, setting, methods: We are presenting the results of ten embryo transfer cycles with low mosaic embryos at Bahceci BIH IVF Center, Sarajevo BIH, between January 2019 – October 2020. All the patients have been informed by a written informed consent form, and had genetic counseling before embryo transfer. Amniocentesis has been performed for all the pregnant women between 14-18 weeks of the pregnancy as prenatal testing.

Main results and the role of chance: Out of the total ten patients, 5 had pregnancy, and the 5 other were not pregnant. The average age of the whole group was; 34.0 ± 5.49 years. The average age of the pregnant women was ; *[3]0.6 ± 5.54 years, and 37.40 ± 2.88 years who were not pregnant*

Limitations, reasons for caution: There are limited number of papers in literature about transferring of the low mosaic embryos. The strength of our presentation is, amniocentesis have been performed as prenatal testing for all the pregnancies.

Wider implications of the findings: There were no correlation between the type of low mosaicism and pregnancy. Since all the pregnant women were 35 years of age or less, It was the only factor which is influencing the pregnancy.We hope that, the results of our cases will improve the management protocols for these cases.

Trial registration number: Not applicable

P-545 Impact of screening for aneuploidies in blastocysts on single embryo transfer live birth rates. A systematic review and meta-analysis

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Study question: Does the selection of blastocysts for single embryo transfer, through the diagnosis of aneuploidy, improves the live birth rate in patients undergoing in vitro fertilization?

Summary answer: There seems to be no statistical difference in live birth rates between embryos with preimplantational genetic diagnosis (PGD) and

Table: Characteristics of Low Mosaicism, The Result of Amniocentesis and the outcome of the pregnancies.

Patient	Age of the patient	Details of Low mosaicism	The result of ET	The Result of Amniocentesis	Outcome of pregnancy
1.	42	47, (+16)	No pregnancy	-	-
2.	33	47, (+6)	Pregnancy	46, XX	Delivery
3.	21	46, (+9, -10)	Pregnancy	46, XX	Delivery
4.	38	45, (-14)	No pregnancy	-	-
5.	35	48 (+4, +15)	No pregnancy	-	-
6.	31	45 (-5)	Pregnancy	46, XY	Delivery
7.	37	47 (+20)	No pregnancy	-	-
8.	35	46, (+15, -3)	No pregnancy	-	-
9.	35	45, (-14)	Pregnancy	46, XY	Ongoing, Second trimester
10.	33	45, (-14)	Pregnancy	46, XX	Ongoing, Second trimester

those without. What is known already: Initial reports indicate that reproductive results improve after the selection of embryos to be transfer after performing a biopsy of the blastomeres, or trophectoderm cells, with the subsequent comprehensive analysis of the chromosomes. However, these results are now questioned. Reports in the literature are contrasting, so the real utility of selecting all embryos through comprehensive chromosome analysis calls for a more careful analysis that compares the risks, costs, and benefits of these techniques and their actual utility in reproductive results of patients treated with in vitro fertilization. Specifically results related to live birth rate.

Study design, size, duration: A systematic review of prospective studies evaluating live birth rate after embryo transfer of embryos selected by blastocyst biopsy for aneuploidy analysis compared with reproductive outcomes in embryo transfers of embryos selected morphologically, without biopsy nor screening for aneuploidies.

Participants/materials, setting, methods: A literature search was performed in PubMed, EmBase, and the Cochrane library (from January 2000 to december 2019). A cumulative meta-analysis and evaluation of heterogeneity was performed for the clinical pregnancy rate. The quality of the included studies was assessed using Cochrane's Risk of Bias tool and ROBINS I for observational studies

Main results and the role of chance: Seven studies were included, three were randomized controlled trials and four were non-randomized studies of intervention (NRSI). The included studies were published between 2013 and 2019. For the preimplantational genetic diagnosis, three studies used array comparative genomic hybridization, three studies used next generation sequencing and only one study used qPCR. A total of 1638 patients were included, only two studies excluded patients with advanced maternal age (>35 years), two studies studied patients with recurrent implantation failure and three studies patients with recurrent pregnancy loss. Regarding the assisted reproduction techniques (ART), only studies where embryos where biopsied after day five for the genetic diagnosis where considered, most used ICSI and performed frozen-thawed transfer of up to two embryos, only one study allowed patients to be transferred with more than two embryos per cycle.

Reproductive outcomes (live birth rate, miscarriage rate, clinical pregnancy) were extracted considering the events per embryo transfer and calculating the pooled odds ratios (OR) with 95% confidence intervals (95%CI) as our main outcome, sensitivity analyses will be performed using the events per cycles to assess the robustness of the effect estimate.

Preliminary meta-analyses resulted in a pooled OR of 1.45 (95%CI 0.24-8.78) for NRSI and 1.34 (95%CI 0.85-2.11) for RCT.

Limitations, reasons for caution: The main limitation was the quantity of studies with acceptable methodology. This generated heterogeneity, hindering the evaluation of the true impact of PGD in ART outcomes. The use of events per embryo transfer as a main outcome could bias the results favoring PGD as less embryos are usually transferred.

Wider implications of the findings: Our results show that there are too few studies with adequate methodology to generate a conclusion about the true benefit of PGD. However, a slight tendency favoring the reproductive outcomes of PGD was found.

Trial registration number: PROSPERO CRD42020198866

P-546 Exome sequencing and preimplantation genetic testing for unexplained recurrent fetal malformations.

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Study question: Could patient suffering unexplained recurrent fetal malformations be benefit of PGT-M by exome sequencing mutations identification?

Summary answer: Patients suffering unexplained recurrent fetal malformations could be benefit of the use of exome sequencing in combination to PGT-M to have a healthy live birth.

What is known already: Fetal malformations account for approximately 3% of live births and causes include: chromosomal abnormalities, exposure to toxic substances or teratogens and infections. Recently, studies have shown that several monogenic diseases are linked to fetal abnormalities. However, because of the large number of potential genes, genetic testing is challenging. Exome sequencing is widely used to detect genetic mutations and has emerged as a useful tool for finding the genetic cause of fetal abnormalities. The aim of this study was to show how exome sequencing in patients suffering unexplained recurrent fetal malformations in combination to PGT-M could lead to successful healthy newborn.

Study design, size, duration: Case report of a non-consanguineous couple with unexplained, recurrent fetal malformations. Couple were recruited during clinical consultation for unexplained recurrent fetal malformations at a private reproductive medicine clinic. The couple had two malformed fetus with the same congenital abnormalities: hydrocephalus, cerebellar vermis agenesis, cerebellar hypoplasia and enlarged cisterna magna. Patients signed written informed consent regarding to exome testing. For fetal sample, informed consent was obtained from parents.

Participants/materials, setting, methods: Sample of the affected fetus were provided. Parental genomic DNA was extracted from peripheral blood. Exome sequencing was performed using TrusightOne (Illumina®). FASTAQ data were processed through BWA and GATK algorithm. VCF files were analysed using Variant Interpreter software. After genetic counselling, PGT-M was performed using linkage polymorphic markers analysis and mutation sequencing. Embryo biopsy was carried at blastocyst stage. Embryos were vitrified and one healthy embryo was thaw and transfer in a subsequent cycle.

Main results and the role of chance: An homozygous novel pathogenic mutation c.641 C>T (p.Ala214Val) in FVLCR2 gene was found. The parents were heterozygous carriers revealing that the detected variant follow an autosomal recessive pattern. The *FLVCR2* (14q24.3) gene encodes a transmembrane protein that belongs to the major facilitator superfamily of secondary carriers that transport small solutes in response to chemiosmosis ion gradients, such as calcium. Mutations in this gene are related to fetal central nervous system defects. This disorder is diagnosed prenatally and is lethal. PGT-M was recommended during genetic counselling. After control ovarian stimulation 14 oocytes were retrieved and finally 4 embryos were suitable for embryo biopsy at blastocyst stage. One embryo was diagnosed as healthy, two affected and one heterozygous carrier. The healthy embryo was thaw and transferred and a healthy male baby was born.

Limitations, reasons for caution: Exome sequencing has technical limitations: only covers mutations in coding regions and does not cover noncoding regions of the genome. It also cannot reliably detect copy-number variants at single gene level.

Wider implications of the findings: This study offers strong evidence of exome-sequencing as a new diagnostic strategy and powerful tool discovering the underlying etiology of recurrent fetal malformations and identifying new genes important for human development. Using this strategy in combination with PGT-M, clinicians can help couples with recurrent fetal malformations to have healthy newborns.

Trial registration number: Not applicable

P-547 Single-cell RNA sequencing identifies molecular regulations associated with poor maturation performance on rescue in vitro matured oocytes

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Study question: What is the transcriptome signature associated with rescue*in vitro*matured (rIVM) oocytes?

Summary answer: GATA-1/CREB1/WNT signaling axis was repressed in rIVM oocytes of poor quality.

What is known already: rIVM aims to produce mature oocytes (MII) for *in vitro* fertilization (IVF) through IVM of immature oocytes collected from stimulated ovaries. It is less popular due to limited success rate in infertility treatment. Genetic aberrations, cellular stress, and the absence of cumulus cell support in oocytes could account for the failure of rIVM.

Study design, size, duration: We applied single-cell RNA sequencing (scRNA-seq) to capture the transcriptomes of human *in vivo* (IVO) oocytes (n = 10) from 7 donors and rIVM oocytes (n = 10) from 10 donors, followed by

studying the maternal age effect and ovarian responses on rIVM oocyte transcriptomes.
Participants/materials, setting, methods: Human oocytes were collected from donors aged 28-41 years with a body mass index of <30. RNA extraction, cDNA generation, library construction and sequencing were performed in one preparation. scRNA-seq data were then processed and analyzed. Selected genes in therIVM vs. IVO comparison were validated by quantitative real-time PCR.
Main results and the role of chance: The transcriptome profiles of rIVM/IVO showed distinctive differences. A total of 1559 differentially expressed genes (DEGs, genes with at least two-fold change and adjusted $p<0.05$) were found to be enriched in metabolic processes, biosynthesis, and oxidative phosphorylation. Among these DEGs, we identified a repression of WNT/ -catenin signaling in rIVM when compared with IVO oocytes. We found that estradiol level exhibited a significant age-independent correlation with the IVO mature oocyte ratio (MII ratio). rIVM oocytes with higher MII ratio showed over-represented cellular processes such as anti-apoptosis. To further identify targets that contribute to the poor outcomes of rIVM, we compared oocytes collected from young donors with high MII ratio versus donors of advanced maternal age and revealed *CREB1*was an important regulator in rIVM. Our study identified GATA-1/CREB1/WNT signaling was repressed in both rIVM condition and rIVM oocytes of low-quality.
Limitations, reasons for caution: In the rIVM oocytes of high- and low-quality comparison, the number of samples was limited after data filtering with stringent selection criteria. For the oocyte stage identification, we were unable to predict the presence of oocyte spindle so polar body extrusion was the only indicator.
Wider implications of the findings: This study showed that GATA-1/CREB1/WNT signaling and antioxidant actions were repressed in rIVM condition and was further downregulated in rIVM oocytes of low-quality, providing us the foundation of subsequent follow-up research on human subjects.
Trial registration number: not applicable

P-548 High-risk genetic matching on gamete donors: complete genes analysis or genotyping test?

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Study question: What carrier screening test is better to reduce the risk of offspring being affected by recessive diseases when genetic matching is performed with gamete donors: complete or targeted genes analysis?
Summary answer: The use of complete genes analysis in the carrier screening of gamete donors reduces the risk of offspring being affected by recessive diseases.
What is known already: Legislative measures and scientific societies alike call for more research to be conducted into recessive diseases in gamete donors, in order to reduce reproductive risk. However, it is still unclear which genes should be studied and what type of data analysis, targeted or nontargeted, should be performed.
Study design, size, duration: This descriptive observational study of 923 oocyte donors and 895 semen donors was conducted from January 2017 to August 2020, at a private gamete bank.
Participants/materials, setting, methods: 1818 gamete donors screened by NGS and nontargeted analysis of the variants, the pathogenic variants detected were analysed to estimate the probability of high-risk genetic matching and to determine the results that would have been obtained if the three most commonly used genotyping tests for carriers of recessive diseases in ART had been applied.
Main results and the role of chance: The probability of high-risk genetic matching with gamete donation, screened by NGS and complete genes analysis, was 5.48%, versus the 0.57-2.8% that would have been obtained if the genotyping test had been applied. Of the 1739 total variants found, only 28.69% would have been detected by all three targeted tests considered and 45.66% of the variants would not have been detected by any of them.
Limitations, reasons for caution: The study was not based in the general population, was limited to a population of Mediterranean ethnic origin. In addition, our study only analysed 302 recessive diseases of the 1,300 plus that have been described.
Wider implications of the findings: Our study highlights the considerable heterogeneity of the genotyping tests commonly used in ART, which present significant differences in their ability to detect pathogenic variants. Therefore, the use of genotyping tests for genetic matching is associated with a higher reproductive risk, compared to the use of complete genes analysis.
Trial registration number: not applicable

P-549 What trophectoderm cells from mosaic embryos tell us about embryonic competence at the transcriptional level

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Study question: Does transcriptome of remaining trophectoderm (TE) reflect the developmental potential of mosaic blastocysts after preimplantation genetic testing for aneuploidy (PGT-A)? Summary answer: TE from low-degree mosaic (Low-mos) and high-degree mosaic (High-mos) blastocysts are transcriptionally equivalent, standing between euploid and aneuploid categories and displaying key deregulated developmental processes.
What is known already: Blastocysts classified as mosaic by PGT-A are associated with lower implantation and higher miscarriage rates than those classified as euploid, yet they still lead to healthy babies. Unveiling the true developmental identity of these embryos faces a dilemma: understanding to which extent they represent technical artefacts or whether they hold own potential to implant and give rise to normal pregnancies. Current RNA sequencing (RNA-seq) techniques allow for the determination of whole transcriptomic profiles even from single cells, which paves the way for the identification of new molecular keys of embryonic competence.
Study design, size, duration: Prospective study comparing RNA-seq data of remaining TE from blastocysts classified as euploid (n=4), Low-mos (n=5), High-mos (n=4) and aneuploid (n=6) by PGT-A. Participants were recruited between October 2018 and November 2019 at IVI-RMA Valencia.
Participants/materials, setting, methods: Chromosomal mosaicism was defined in the range 30%-<50% (Low-mos) and 50%-<70% (High-mos) using a next-generation sequencing (NGS) validated algorithm. Whole TE fractions were separately collected and processed for RNA-seq. Differentially expressed genes (DEGs) were calculated with DESeq2 package [Benjamini-Hochberg (BH)-adjusted p<0.01 & abs(log2FoldChange)>2 significant]. Fgsea algorithm was used for enrichment analysis on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms (BH-adjusted p<0.01 significant).
Main results and the role of chance: For comparisons, TE from euploid blastocysts were used as control. At the gene level, 15 DEGs were found in Low-mos, 20 DEGs in High-mos, and 64 DEGs in aneuploid blastocysts. To address the functional implications of these differences, pathways significantly deregulated according to KEGG and GO categories were identified. TE from aneuploid blastocysts displayed significant downregulation in up to 115 KEGG and GO processes directly involved in processing and integrity maintenance of nuclear and mitochondrial genomes, a reflection of their aberrant chromosomal identity. In addition, TE from High-mos and Low-mos were transcriptionally equivalent (0 DEGs between both groups), with 23 overlapping KEGG and GO processes significantly downregulated compared with control. Importantly, main significantly-affected processes included mitotic sister chromatid segregation, NIK NF-kB activity, regulation of apoptosis, and pathways related to the biosynthesis and metabolism of proteins, fatty acids, carbohydrates and steroid hormones. These findings indicate that mosaic embryos comprise a unique developmental entity, which swims between the euploid and aneuploid waterfronts and may regulate survival by diverse mechanisms, including cell proliferation and apoptosis.

Limitations, reasons for caution: This is a descriptive, single-center study with limited sample size. TE fractions were obtained by micromanipulation, which may have led to potential cross-contamination with the inner cell mass.
Wider implications of the findings: Transcriptomic equivalence between Low-mos and High-mos TE fractions questions the biological significance of inferring mosaicism degrees from single biopsies. Deregulated processes in these embryos support their reduced developmental and live birth potential, pointing to mechanisms that may mediate survival in the presence of aneuploid cells, as shown in the mouse.
Trial registration number: Not applicable

P-550 Clinical outcomes of mosaic embryos are similar between young and older women.

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Study question: Are there differences in the clinical outcomes of mosaic embryos depending on the female age?
Summary answer: Clinical outcomes of mosaic embryo transfers are similar regardless female age.
What is known already: Chromosomal abnormalities are common in embryos analyzed in preimplantation genetic testing for aneuploidy (PGT-A) cycles. Mosaicism (the presence of two or more chromosomally distinct cell lines) is a usual event in embryos derived from IVF cycles.

Several studies show that mosaic embryos have reduced potential to reach term, compared to euploid embryos. The factors affecting the implantation potential and development of mosaic embryos are controversial. Recently, Victor et al. (2019) argued that mosaic blastocysts generated at younger ages show better outcomes compared to older ages. The aim of this study was to test this hypothesis in our centre.
Study design, size, duration: A total of 136 mosaic embryos from patients undergoing PGT-A cycles from May 2014 to October 2020 were retrospectively analyzed in this study. The blastocyst trophoectoderm biopsies of day 5 and 6 were analysed by aCGH (n=47, 30.1%) and NGS (n=109, 69.9%). An embryo was considered mosaic when the percentage of aneuploid cells were 25-50% in aCGH and 20-50% in NGS. Only single embryo transfer cycles were included in the analysis.
Participants/materials, setting, methods: Embryo analysis were performed using Agilent SurePrint G3 8x60K CGH microarrays or Veriseq NGS (Illumina), with previous whole genome amplification. We evaluated if clinical results of mosaic embryos transfers in IVF cycles were correlated with female age. The main outcome measures were β-hCG, implantation rate and ongoing pregnancy rate. β-hCG was measured in blood 14 days after the embryo transfer and was considered positive when it was >2 mUI/ml.The statistical analysis was performed with SPSSv20.0.
Main results and the role of chance: A total of 136 mosaic embryos were included in this study. Overall, we evaluated factors affecting embryo mosaicism implantation potential. Neither the percentage of mosaicism nor the segmental mosaicism were related to mosaic embryo implantation, pregnancy and ongoing pregnancy rates.

To evaluate the impact of female age in clinical outcomes, we established two different groups depending on whether mosaic blastocysts were generated from oocytes retrieved at young maternal ages (≤35 years; n=62) or at older ages (>35years; n=74).

No differences were found between groups. Nonetheless, to reduce bias, embryo quality, percentage of mosaicism, segmental mosaicism and whether the transferred embryo was frozen or fresh were included as confounding factors.

The rate of positive -hCG was similar between groups: 45.2% in ≤35y group vs 54.1% in >35y (p=0.476). The implantation rate was also similar: 30.6% vs 39.2% % (p=0.855), respectively. Furthermore, the ongoing pregnancy rate was higher in the >35y group (35.1%), compared to the ≤35y group (19.4%) without reaching statistically significant differences (p=0.245).
Limitations, reasons for caution: The sample size is a limitation. aCGH test and a different definition for mosaic embryo in terms of percentage of abnormal cells was employed in this study compared to Victor et. al. (2019) study. Larger prospective studies should evaluate the impact of maternal age in the outcome of mosaic embryos.
Wider implications of the findings: Our results challenge that female age is associated with clinical outcomes after the transfer of mosaic embryos. Comparable results were obtained in young and older women. Therefore, in the absence of euploid embryos, mosaic embryos might be considered for transfer and similar outcomes are expected regardless of the maternal age.
Trial registration number: Not applicable

P-551 Blastocyst cohort size is not associated with embryo aneuploidy: comprehensive multi-centre data from current preimplantation genetic testing cycles

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Study question: Does blastocyst cohort size impact aneuploidy rates, evaluated by next generation sequencing (NGS)?
Summary answer: Embryo aneuploidy rates were independent of blastocyst cohort size across all patient ages.
What is known already: The effects of ovarian response on oocyte and embryo quality remain controversial. Several studies have proposed that a high response to ovarian stimulation may negatively impact oocyte competence. Alternatively, irrespective of maternal age, a poor ovarian response may potentially compromise embryo quality. Using blastocyst cohort size as an indirect measure of ovarian response, previous studies applying array comparative genomic hybridisation (aCGH) have demonstrated that the number of embryos available for biopsy does not impact embryo aneuploidy rates. Nevertheless, these findings remain to be confirmed in a comprehensive cohort, using current approaches for preimplantation genetic testing for aneuploidies (PGT-A).
Study design, size, duration: Retrospective, international, cohort study of 3998 patients from 16 clinics undergoing PGT-A from 2016-2020. We evaluated 11665 blastocysts, tested using trophectoderm (TE) biopsy and next generation sequencing (NGS). To eliminate bias of multiple treatments, we considered only the first PGT-A cycle for all patients. Both autologous and donation cycles were included in the analysis. Cycles were excluded if they utilised preimplantation genetic testing for monogenic disorders (PGT-M) or preimplantation genetic testing for structural rearrangements (PGT-SR).
Participants/materials, setting, methods: We evaluated aneuploidy and mosaicism rates, as well as the proportion of patients who had at least one euploid embryo suitable for transfer. Findings were stratified according to SART-defined maternal age groups, <35 (n=698/2622 patients/blastocysts), 35-37 (n=988/3141 patients/blastoycsts), 38-40 (n=1447/3939 patients/blastocysts), 41-42 (653/1562 patients/blastocysts) and >42 (212/401 patients/blastocysts) and blastoycst cohort size (1-2, 3-5, 6-9 and 10 or more biopsied blastocysts).
Main results and the role of chance: The mean maternal age was 37.0±3.7. The overall embryo aneuploidy rate was 50.6% (5904/11665), while mosaicism was established in 4.0% (469/11665) of blastocysts. As expected, the proportion of aneuploid embryos increased steadily with advancing maternal age (31.8%, 41.5%, 58.4%, 71.2%, 87.8%; p<0.0001), while mosaicism rates did not vary significantly (p=0.2). Within each age group, we observed no association between the number of blastocysts biopsied and aneuploidy or mosaicism rates. However, as previously suggested, the chance of having at least one euploid embryo increased linearly with the number of embryos biopsied. We observed that young patients (<35) with 1-2 blastocysts had a 70.4% of having at least one embryo suitable for transfer, which increased to 96.4% and 99.2% with 3-5 and 6-9 blastocysts, respectively. Similar trends were observed in the 36-38 and 39-40 age groups. Patients in the 40-41 age group had a significantly lower chance of having a suitable embryo for transfer. Nevertheless, the chance increased from 27.2% with 1-2 embryos to 61.2% with 3-5 blastocysts. Patients with >10 embryos had at least one euploid embryo in 100% of cases, across all ages.

Albeit, the numbers of patients within this category was low, and decreased significantly with advancing maternal age.
Limitations, reasons for caution: While blastocyst cohort size is considered to be an indirect measure of ovarian reserve, the number of oocytes retrieved was not evaluated. Our study only included the first PGT-A cycle for all patients. Subsequent, alterations in stimulation protocols may have resulted in an improved response in some patients.
Wider implications of the findings: The comprehensive nature of the study, based on current PGT-A approaches and a large number of cycles across 16 centres increases clinical confidence in the notion that ovarian response is independent of embryo aneuploidy. Importantly, our findings may serve as a valuable clinical resource to guide patient counselling strategies.
Trial registration number: NA

P-552 Delayed blastocyst development is associated with a higher risk of aneuploidy in patients of advanced maternal age

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Study question: Is delayed blastocyst development, assessed by the day of trophectoderm (TE) biopsy, associated with higher rates of aneuploidy?
Summary answer: Our findings show an association between delayed blastocyst development and poorer prognosis, in terms of euploidy rates, in patients of advanced maternal age.
What is known already: Extended culture of embryos past day 5 of development has become routine practice in all freeze-all cycles, including those applying preimplantation genetic testing for aneuploidies (PGT-A). As healthy live births have been obtained from day 6 and day 7 blastocysts, increasing the pool of embryos available for PGT-A is beneficial, particularly for patients of advanced maternal age who face higher cancellation rates. Nevertheless, the association between delayed blastocyst development and aneuploidy rates remains unclear. As current studies have reported opposing findings, detailed analysis of the chromosomal constitution of slowly developing embryos remains paramount.
Study design, size, duration: Retrospective, international, multicentre cohort study of 4211 patients undergoing preimplantation genetic testing for aneuploidy (PGT-A) from January 2016 to July 2020. We evaluated the chromosomal status of 14757 blastocysts tested using TE biopsy and next generation sequencing (NGS). Both autologous and donation cycles were included in the analysis. Cycles were excluded if they utilised preimplantation genetic testing for monogenic disorders (PGT-M) or preimplantation genetic testing for structural rearrangements (PGT-SR).
Participants/materials, setting, methods: We evaluated euploidy, aneuploidy and mosaicism rates reported in day 5 (n=9560), day 6 (n=4753) and day 7 (n=262) blastocysts, stratified by SART-defined maternal age categories (<35, 35-37, 38-40, 41-42, >42). We further assessed the type and frequency of abnormalities reported in all blastocysts classified as clinically unsuitable, according to the day of biopsy. Finally, we examined the specific chromosomes affected in embryos diagnosed with a single uniform (n=3882) or single mosaic (n=518) abnormality.
Main results and the role of chance: The mean maternal age within our patient cohort was 39.9±3.7. Overall, slowly developing blastocysts were significantly more likely to be classified as clinically unsuitable (60.6%) compared to day 5 embryos (55.2%; p< 0.0001). This correlation was also observed when stratified by age, with the exception of the <35 age group (p=0.25). Markedly, the risk of aneuploidy in slowly developing blastocysts became progressively higher with advancing maternal age (p<0.0001). We did not observe any significant differences in the types of abnormalities diagnosed in slowly developing embryos compared to day 5 blastocysts. Nevertheless, abnormalities affecting all chromosomes were present at the blastocyst stage. Single trisomies and monosomies were the most frequent across all age groups, and were equally prevalent in day 5, 6 and 7 blastocysts. These most commonly affected chromosomes 16, 22, 21 and 15. We observed no significant differences in the incidence of segmental aneuploidies in relation to the day of biopsy, across all age groups. When considered separately, day 7 blastocysts presented with higher rates of structural aberrations, however low numbers limited statistical power. Finally, delayed blastocyst development was not associated with higher mosaicism rates (p=0.79). Interestingly, single mosaic trisomies and monosomies were most frequently associated with chromosome 19.
Limitations, reasons for caution: Due to the retrospective nature of the study, full elucidation of all potential confounders may not be possible in all instances. The low number of day 7 blastocysts limited statistical power. As such, the results from day 6 and day 7 embryos were evaluated together.
Wider implications of the findings: Our findings offer an important clinical resource for counselling patients of advanced maternal age. Maternal aging may be associated with a higher incidence of aneuploidy in slowly developing blastocysts. Nevertheless, extended culture increases the pool of biopsiable blastocysts, ultimately improving the chance of having a euploid embryo for transfer.
Trial registration number: NA

P-553 Women with molar pregnancies have a genetic susceptibility to aneuploid miscarriages

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Study question: What causes non-molar miscarriages in women with one hydatidiform mole (HM)?
Summary answer: We found a higher rate of aneuploidies in the non-molar miscarriages of women with HM than in those from women with sporadic or recurrent miscarriages.
What is known already: Women with hydatidiform moles have higher rates of miscarriages and women with recurrent miscarriages have higher rates of moles than women from the general population.
Study design, size, duration: We retrieved archived formalin-fixed paraffin embedded tissues from non-molar miscarriages of patients with one HM and analyzed them for the presence of aneuploidies using single nucleotide polymorphism (SNP)-microarray. We next determined the meiotic origin of the aneuploidies by genotyping the aneuploid non-molar miscarriages along with the parental genomes using microsatellite markers.
Participants/materials, setting, methods: All participants and some of their partners provided written consent to participate in our study, agreed to a blood draw for genotyping analysis, and agreed for us to retrieve their molar and non-molar tissues from various histopathology laboratories for research purposes.
Main results and the role of chance: We demonstrate for the first time that patients with an HM and miscarriages are at higher risk for aneuploid miscarriages [83.3%, 95% confidence interval (CI): 0.653–0.944] than women with sporadic (51.5%, 95% CI: 50.3–52.7%, p value = 0.0003828) or recurrent miscarriages (43.8%, 95% CI: 40.7–47.0%, p value = 0.00002). Genotyping the aneuploid miscarriages and the parental genomes demonstrated that most of the aneuploidies originated from errors in maternal meiosis I or II.

Limitations, reasons for caution: We were able to retrieve only 30 non-molar miscarriages from women with one HM for analysis. Expanding such analysis to a larger and independent cohort of miscarriages from such patients will be important to validate our observations.
Wider implications of the findings: Our data suggest common genetic female germline defects predisposing to HM and aneuploid non-molar miscarriages in some patients.
Trial registration number: not applicable

P-554 Reproductive risks and preimplantation genetic testing intervention for X-autosome translocation carriers

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Study question: For X-autosome translocation [t(X-A)] carriers, is it a more applicable preimplantation genetic testing (PGT) strategy, that distinguishing non-carrier from euploid/ balanced embryos and prioritized transfer?
Summary answer: Noncarrier and carrier embryos discrimination in PGT is an applicable strategy to avoid transferring genetic and reproductive risks to the offspring of t(X-A) carriers.
What is known already: Balanced t(X-A) is a specific reciprocal translocation, with a higher risk of detrimental phenotype and fertility issues compared to individuals with autosomal translocation. Alternative X-chromosome inactivation (XCI) is a specific pathogenic mechanism in this population. For carrier offspring of couples with t(X-A), the genetic counseling is challenged in both the prenatal and postpartum stages, because of the complexity and severity of phenotype outcomes that are unpredictable and associated with the complex XCI mechanism. Therefore, caution is necessary when designing a PGT strategy for couples with t(X-A).
Study design, size, duration: A retrospective study. We collected a 3-year-old girl with maternal translocation 46,X,t(X;1)(q28;p31.1) presenting with multiple congenital disabilities. Three couples with female t(X-A) carrier requesting for PGT.
Participants/materials, setting, methods: Karyotype analysis, whole-exome sequencing (WES), and X inactivation analysis were performed for the girl with congenital cardiac anomaly, language defect, and mild neurodevelopmental delay. PGT based on next-generation sequencing following the microdissecting junction region to distinguish noncarrier and carrier embryos were used in three couples with female t(X-A) carrier (Cases 1-3).
Main results and the role of chance: The girl carried a maternal balanced translocation 46,X,t(X;1)(q28;p31.1). WES revealed none monogenic mutation related to her phenotype, but she carried a rare skewed inactivation of the translocation X chromosome and spread to the adjacent interstitial 1p segment, contrary to her mother. All translocation breakpoints of Cases 1-3 were successfully identified and each couple underwent one PGT cycle. Thirty oocytes were retrieved, and 13 blastocysts were eligible for biopsy, of which 6 (46.15%) embryos were balanced and only 4 were noncarriers. Three frozen embryo transfers with noncarrier embryos resulted in the birth of two healthy children (one girl and one boy), who were subsequently confirmed to have normal karyotypes. We reported a girl with multiple congenital disabilities resulting from maternally balanced t(X-A) and validated that noncarrier and carrier embryo discrimination is an effective and applicable strategy for avoiding transferring genetic and reproductive risks to the offspring from t(X-A) carriers.
Limitations, reasons for caution: Here, we reported a girl with multiple congenital disabilities resulting from maternally balanced t(X-A) found different XCI patterns, while we did not further determine the mechanism causing the different XCI patterns between the girl and her mother.
Wider implications of the findings: We demonstrated passing on a balanced t(X-A) may result in clinical manifestations associated with the X-inactivation, and verified the PGT strategy, that distinguishing normal and carrier embryos in can widely applied in t(X-A) carrier couples to avoid the genetic and reproductive risk of transferring t(X-A) to the next generation.
Trial registration number: the National Key Research & Developmental Program of China (2018YFC1004900), the National Natural Science Foundation of China (81771645 and 81971447), the Key Grant of Prevention and Treatment of Birth Defect from Hunan Province (2019SK1012), Hunan Provincial Grant for Innovative Province Construction (2019SK4012) and the Research Grant of CITIC-Xiangya (YNXM-201916).

P-555 Recurrent pregnancy loss is associated with changes in the pre-pregnant endometrial gland transcriptome

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Study question: Do endometrial gland factors influence recurrent pregnancy loss?
Summary answer: The endometrial gland transcriptome during the window of implantation is altered in women with recurrent pregnancy loss compared to controls.
What is known already: Secretions from endometrial glands contribute to the uterine environment that supports the attachment and implantation of the embryo in early pregnancy. Studies have attempted to identify an endometrial gene expression pattern associated with recurrent pregnancy loss however, the cellular heterogeneity within the endometrium may obscure important differences in specific cell populations.
Study design, size, duration: An observational study comparing controls and women with recurrent pregnancy loss.
Participants/materials, setting, methods: Endometrial samples were collected during the implantation period of the menstrual cycle from five matched participant egg donor controls and women with recurrent pregnancy loss. Endometrial glands were isolated from fresh endometrial biopsies and RNA sequencing was performed. A differential gene expression analysis and a gene ontology enrichment analysis was performed between egg donor controls and women with recurrent pregnancy loss.
Main results and the role of chance: This study reports a glandular epithelium specific gene expression profile and demonstrates differential gene expression of endometrial glands from women with recurrent pregnancy loss compared to controls. 18 genes were upregulated and 1 gene was downregulated in the endometrial glands from women with recurrent pregnancy loss compared to controls (5% false discovery rate). Biological processes which contain genes that were differentially expressed in women with recurrent pregnancy loss compared to controls include epithelial cell migration and regulation of secretion by the cell.
Limitations, reasons for caution: This is an observational study with a relatively small sample size.
Wider implications of the findings: This study identified differences in gene expression in women with recurrent pregnancy loss that are specifically associated with endometrial glands rather than endometrium as a whole. These differences could be used to identify a perturbed endometrium, isolate causes of recurrent pregnancy loss and develop targeted therapies.
Trial registration number: not applicable

P-556 NAT10-mediated N4-acetylcytidine in RNA regulates mouse oocyte maturation in vitro

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Study question: Does NAT10-mediated N4-acetylcytidine (ac4C) in RNA, a newly identified mRNA epigenetic modification, participate in modulating in vitro maturation(IVM) of oocytes?
Summary answer: NAT10-mediated ac4C modification is an important regulatory factor during oocyte maturation in vitro, by regulating genes associated with translation, mitochondrial functions and protein destabilization.
What is known already: Unlike somatic cells, transcription and translation are uncoupled during oocyte maturation and gene expression is mainly regulated by post-transcriptional modulation, including mRNA degradation, translation

and posttranslational modification, which are complex and have not been fully investigated. RNA ac4C is a newly identified mRNA modification and a key determinant of post-transcriptional regulation, which has been shown to promote mRNA stability and translation, and NAT10 is the only known RNA acetyltransferase. Therefore, NAT10-mediated ac4C represents a possible epigenetic regulator in oocyte maturation.

Study design, size, duration: Oocytes at different stages from mice were collected to detect the changing levels of ac4C and NAT10 during maturation. NAT10 in GV-stage oocytes was knocked down before IVM, to confirm the regulatory role of NAT10-mediated ac4C in meiotic process, followed by further exploration of cellular mechanisms. Each experiment was repeated at least three times, and data were analyzed by chi-square test, one-way ANOVA or unpaired-sample t-test.

Participants/materials, setting, methods: The expression of ac4C and NAT10 was detected by immunohistochemistry. NAT10 was knocked down in GV-stage oocytes by RNA interference through electroporation. The efficacy of knockdown was confirmed by qPCR and immunohistochemistry targeting ac4C and NAT10, and the percentages of oocytes maturated in vitro were compared among groups. High-throughput sequencing and RNA immunoprecipitation were performed to reveal the modulated genes. Proteins specifically binding to ac4C sites were identified by RNA pulldown and mass spectrometry.

Main results and the role of chance: We first retrieved publicly available data from GEO and found that transcripts with potential ac4C sites were enriched in genes downregulated during IVM (P<0.001). The biased distribution of ac4C implicated a possible regulatory role. Then immunohistochemistry revealed significantly decreasing trends of ac4C and NAT10 expression from immature to mature oocytes. With NAT10 knockdown, ac4C modification was reduced and meiotic progression was significantly retarded. Specifically, the rate of first body extrusion was significantly decreased with NAT10 knockdown (34.6%) compared to control oocytes without transfection (74.6%) and oocytes transfected with control siRNA (72.6%) (p<0.001), while rates of germinal vesicle breakdown were not affected (P=0.6531). High-throughput sequencing and RNA immunoprecipitation revealed that the modulated genes were enriched in biological processes known to be associated with oocyte maturation, including translation, mitochondrial translational elongation and termination, and protein destabilization. Also, we identified a series of proteins specifically binding to ac4C probes by RNA pulldown and mass spectrometry, through which ac4C modification may exert its function in post-transcriptional modulation.

Limitations, reasons for caution: This study was performed in vitro. The role of NAT10-mediated ac4C in vivo remains to be elucidated. Also, limited by current techniques, ac4C modification in oocytes cannot be detected. Our exploration of regulated genes and ac4C binding proteins were performed in somatic cell lines.

Wider implications of the findings: Post-transcriptional modulation is crucial in oocyte maturation. Our study using in-vitro systems for mouse oocyte identified NAT10-mediated ac4C as an important regulator in IVM. It provided a new insight into the epigenetic mechanisms of IVM, which may lead to improvement of clinical IVM systems.

Trial registration number: not applicable

P-557 Trophectoderm biopsy technique and rate of mosaicism in human blastocysts

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Study question: Do different trophectoderm biopsy techniques affect mosaicism rate in human blastocysts?

Summary answer: No statistical significance was found between biopsy techniques and mosaicism rate. However, an increase in mosaicism rate was observed when the flicking technique was used.

What is known already: Mosaicism is defined as two or more distinct cell lines within an embryo. Recent advances in Next Generation Sequencing (NGS) technology with an increased sensitivity allows a higher accuracy in quantification of mosaic levels in biopsied cells. The incidence of mosaicism is widely debated as there are many attributing technical and biological factors. Since, trophectoderm biopsy is a technically challenging process, it is crucial to ensure that the both biopsied cells and blastocyst suffers minimal damage during biopsy.

Study design, size, duration: This is a prospective study involving 222 patients (age range= 18-44, mean age= 31.5) who underwent IVF cycles in Alpha IVF, Malaysia from March 2019 to August 2019. Six hundred and sixty-eight (668) of the blastocysts were biopsied on Day 5 (Group 1) while 177 blastocysts were biopsied on Day 6 (Group 2). The blastocysts in these groups were further categorised into their corresponding biopsy techniques: (A) laser+pulling; (B) laser+flicking; (C) flicking only.

Participants/materials, setting, methods: Blastocysts which were at least fair graded (Gardner, 1999) were biopsied and vitrified (Cryotec, Japan). The number of biopsied cells ranged from 5 to 10 cells. All biopsied trophectoderm samples were subjected to Preimplantation Genetic Testing for Aneuploidy (PGT-A) with Next Generation Sequencing (NGS) (Ion Torrent, USA). Chromosomal mosaicism analysis was done using ReproSeq Mosaic PGS w1.1 workflow. Trophectoderm biopsied sample which were tested to have 20% to 80% aneuploid cells were reported as mosaic.

Main results and the role of chance: In Group 1, the mosaicism rates for biopsy technique A, B and C were 23.3% (104/446), 28.2% (58/206) and 37.5% (6/16) respectively. In Group 2, the mosaicism rates for biopsy technique A, B and C were 14.6% (7/48), 19.5% (23/118) and 27.3% (3/11) respectively. There were no significant differences (p>0.05) in mosaicism rates between all study groups and subgroups.

Limitations, reasons for caution: Although no statistical significance was found between trophectoderm biopsy techniques and the prevalence of mosaicism, there is a trend of an increase in mosaicism rate when the flicking technique was used. Therefore, further studies with a larger sample size should be undertaken.

Wider implications of the findings: Our study demonstrates a trend in the decrease of mosaicism rate when laser pulses was used to loosen the cell junction of targeted cells. Hence, in place of the flicking method alone, laser pulses should be applied during trophectoderm biopsy if our findings are confirmed in a larger controlled study.

Trial registration number: Not Applicable

P-558 SNP array versus karyotype for prenatal diagnosis in fetuses with abnormal ultrasound: a systematic review and meta-analysis

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Study question: Does Single nucleotide polymorphism (SNP) array provide a diagnostic advantage over conventional karyotype in prenatal diagnosis for fetuses with an abnormal ultrasound?

Summary answer: SNP array in the prenatal setting provides an incremental diagnostic yield over karyotype and the diagnostic accuracy is comparable with combined SNP array and karyotype

What is known already: Single nucleotide polymorphism and comparative genomic hybridization based arrays (aCGH) are the two chromosomal microarray (CMA) platforms available. Guidelines which recommend offering CMA instead of karyotyping for prenatal diagnosis are mainly based on studies that compared aCGH with karyotype. There is a paucity of reviews that critically appraise the role of SNP array as a prenatal diagnostic tool. We decided to estimate the incremental yield of SNP array over karyotype in detecting chromosomal abnormalities, and to determine the diagnostic accuracy of SNP alone compared with SNP array and karyotype in combination for prenatal diagnosis in fetuses with an abnormal ultrasound.

Study design, size, duration: We conducted a systematic review of studies comparing SNP array with karyotype for prenatal diagnosis in fetuses with an abnormal ultrasound. We performed a literature search in the electronic databases of EMBASE, PubMed, CENTRAL, CDSR, SCOPUS and Web of science for relevant studies published in the English language between January1996 and May 2020. We also hand searched the referenced list of included studies and performed a google search for grey literature to identify potential studies.

Participants/materials, setting, methods: The study population was women undergoing prenatal diagnosis for abnormal fetal ultrasound. Studies in which SNP array and karyotyping had been used in fetuses with abnormal ultrasound and which allowed for a 2 x 2 data extraction table were included. We estimated the incremental yields for SNP array over karyotype. For determining the diagnostic accuracy, we considered SNP array alone as the index test and combined karyotype & SNP array as the reference standard.
Main results and the role of chance: We included six studies for quantitative analysis. After pooling results, incremental yield of SNP array over normal karyotype was 10% (95% confidence interval, CI 4 to 16%) in fetuses with abnormal ultrasound while incremental yield of karyotype over SNP array was 1% (95% CI 0 to 2%). The agreement between SNP array and karyotype was 92%. Variant of uncertain significance (VUS) rates ranged from 4-8 %.

For SNP array alone versus combined SNP array and conventional karyotype, pooled sensitivity and specificity was 0.96 (95% CI 0.91 to 0.99) and 1.00 (95% CI 0.99 to 1.00) respectively. The Area under curve (AUC) was 0.99 indicating the discriminating ability of the SNP array was very high to identify the fetus with chromosomal abnormalities.
Limitations, reasons for caution: Only studies published in English were included. There was some degree of heterogeneity in inclusion criteria for the included studies.
Wider implications of the findings: The current study suggests SNP array alone can replace conventional karyotype for prenatal diagnosis in fetus with an abnormal ultrasound. Limitations to adoption of SNP testing might include the requirement of requisite expertise to interpret the results and counsel patients appropriately, especially with the propensity of SNPs to identify VUS.
Trial registration number: Not applicable

P-559 Proof of principle for Extended Carrier Screening (ECS) in medically assisted reproduction: First 33 cases of genetic matching for donors and recipients

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Study question: Does implementation of ECS to reduce reproductive risk when using a donor with a known carrier status serves as a wider model for ART patients.
Summary answer: ECS should be routinely offered to ART patients using their own or donor gametes to reduce risk of having a child with a recessive condition.
What is known already: Responsible implementation of ECS in assisted reproduction is required as commercial offerings increase and become more accessible; ESHRE ethical guidelines are shortly to be published after consideration of stakeholder reviews. Increasingly ECS is included in donor screening, rejecting potential donors for a known carrier status will reduce donor gamete availability. Clinics should consider potential match yield in carrier panels to develop tools and specialist support to deliver and guide patients to help make informed decisions for ECS.
Study design, size, duration: Retrospective evaluation of ECS results for the first 33 patients who undertook counter screening in the clinic setting from April 2020 to December 2020 before using a donor with known carrier status. The findings would serve as proof of concept for wider application.
Participants/materials, setting, methods: Patients had opted to undertake ECS after discussion of risk estimates, family history review and personal options. Testing was commissioned between two ECS providers for international donor banks using panels of 283+ genes. Incidence of carrier match, the number of variants reported, and their clinical significance were reviewed. Main results and the role of chance: Of the 33 co-carrier tests, one carrier match for a patient and potential donor was identified. 41%, 26%, 9% and 3% of patients were carriers of 1, 2, 3 and 4 pathogenic variants respectively in 30 different genes. In 21% of patients, no pathogenic variants were reported. Of the variants identified as incidental findings, six were actionable and eligible for cascade screening to the wider family. This included Cystic Fibrosis, Sickle cell and Thalassemia. A variant for Familial hypercholesterolemia had preventative value. An incidental finding of a fragile X pre-mutation allowed for PGT-M as part of planned treatment
Limitations, reasons for caution: Findings from this first cohort of 33 tests may not represent the general population, follow up evaluation as participant numbers increase is required.
Wider implications of the findings: Implementation of guidelines is required to ensure consistency of methodology and availability of transparent information for ECS to ART patients. Incidental findings may be of value to the patient and wider family.
Trial registration number: not applicable

P-560 Comparative analysis of non-invasive preimplantation genetic testing of aneuploidies (niPGT-A), PGT-A and IVF cycles without aneuploidy testing: preliminary results

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Study question: Can non-invasive preimplantation genetic testing of aneuploidies (niPGT-A) improve the clinical outcome in IVF patients after proper validation?
Summary answer: We demonstrate the usefulness of the embryonic cell-free DNA (cfDNA) in the blastocyst culture medium to select more objectively the blastocysts with higher implantation potential.
What is known already: One of the greatest challenges in IVF is accurately selecting viable embryos that are more likely to achieve healthy livebirths following embryo transfer. Trophectoderm (TE) biopsy and PGT-A provide a direct assessment of chromosome status and improve implantation and clinical pregnancy rates per transfer. A non-invasive alternative is to analyse embryonic cfDNA in the blastocyst culture medium. Previous studies have shown that cfDNA testing in culture medium of blastocysts on day 6 of development allows aneuploidy detection with high concordance rates compared to TE biopsy and inner cell mass (Rubio et al., 2020).
Study design, size, duration: Observational study of the clinical application of niPGT-A (July 2020-December 2020). The clinical application consisted in a first validation phase, comparing TE biopsies with cfDNA in the media of 28 blastocysts. And, in a second phase, niPGT-A was applied and the outcome of 13 single embryo transfers (SETs) compared to 13 PGT-A SETs and 130 IVF/ICSI SETs performed in a period of six months. In the three groups, women and donors age was ≤38 years.
Participants/materials, setting, methods: Embryos were cultured in a Geri incubator (Merck) up to day 4, and then individually cultured in 10µl drops of CCSS (Fujifilm) until day 6 in a bench-top K-system. At day 6, blastocysts were vitrified, and media collected in sterile PCR tubes after at least 40 hours in culture. After collection, media were immediately frozen and analyzed by NGS analysis in our reference laboratory (Igenomix, Spain). Deferred transfer was performed according to media results.
Main results and the role of chance: Before the first clinical cases, a validation of the protocol comparing the results of cfDNA with the TE biopsies of the same day-6 blastocyst was performed, and ploidy concordance rates were 87.5%.

Similar results were found for niPGT-A and PGT-A in terms of aneuploidy results and in clinical outcomes. The percentages of informative results were 95% and 97% and the aneuploidy rates were 44% and 46%, for niPGT-A and PGT-A, respectively. Clinical pregnancy rates were in both groups of aneuploidy testing, 69.2%, with 8 ongoing pregnancies (61.5%) and 4 tested by prenatal screaning NACE. For untested embryos clinical pregnancy (57.7%) and ongoing pregnancy rates (48.5%) were lower than in the two groups of tested embryos (niPGT-A and PGT-A).

In the niPGT-A cycles embryo transfer was performed according to media results and morphology. We did a secondary analysis of which blastocyst we would transfer, if only morphology is considered. We observed that if we only select the embryos by morphology, in 61.5% of the cases we would choose the same embryo than with niPGT-A, and in 30.4% of the cases we would transfer a blastocyst with an aneuploid medium.
Limitations, reasons for caution: Our results are encouraging but should be interpreted with caution due to the small sample size. Larger and randomized controlled trials are needed to verify and extend our findings in each group.

Wider implications of the findings: We observed consistent results for niPGT-A compared to TE biopsies in our internal validation. These results endorse the clinical application of niPGT-A in the routine of the laboratory and can avoid the embryo manipulation also reducing the subjectivity when embryos are selected only by morphology.
Trial registration number: Sa-16552/19-EC:428

P-561 Male and female blastocyst: any difference other than the sex?

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Study question: Is there any imbalance in the sex ratio (SR) and in the aneuploidy rate of male and female human blastocysts from a PGT-A programme?
Summary answer: Although SR in human blastocysts is significantly male-biased, more aneuploidies are observed among male blastocysts, resulting in comparable euploid male and female embryos available.
What is known already: More boys than girls are born worldwide, meaning that the SR at birth is biased towards males. Differences in the SR of children born after ART have been also reported. Factors such as the insemination technique or the day of embryo transfer have been shown to be related to the SR at birth, but whether the SR is shifted during the preimplantation and/or postimplantation development remains unknown. Study design, size, duration: Embryos from patients undergoing 921 PGT-A cycles from September 2017 to February 2020 were included in the study. Data from the chromosomal constitution of 2637 biopsied blastocysts was retrospectively analysed.
Participants/materials, setting, methods: Embryos were cultured in time-lapse incubators with low oxygen tension (5%) (Embryoscope®; Geri®) using single-step medium (Global®, LifeGlobal®; GTL™, Vitrolife). Blastocyst biopsy was performed between D5-D7 followed by immediate vitrification (Cryotop®, Kitazato). Trophectoderm samples were analysed by NGS. Embryos were categorized as euploid, aneuploid or mosaic. Embryos were called as mosaic when the deviation from the normal copy number was ≥30% and <70%.
Main results and the role of chance: Overall biopsies from 2637 blastocysts were analysed, 1320 on day 5 (50.1%), 1169 on day 6 (44.3%) and 148 on day 7 (5.6%). Sex distribution among the embryos analysed was skewed in favor of male sex with 1401 diagnosed as male (53.1%) and 1236 were female (46.9%), [OR (95%CI):1.13(1.05-1.22)]. As a consequence of this biased SR, more male embryos reached the blastocyst stage and were biopsied both on day 5/6 (708/1320, 53.6% on day 5 and 619/1169, 53% on day 6). Embryos biopsied on day 7 were balanced between sexes with 50% being male and 50% being female. Following biopsy and PGT-A, 1086 (41.2%) of the embryos were classified as euploid, 1349 (51.16%) as aneuploid, and 202 (7.7%) as mosaic embryos. More chromosomal anomalies were observed among male blastocysts when compared to the female ones, 738 (52.7%) vs 611 (49.4%). Similarly, mosaicism was more frequents in male as compared with female blastocysts, 123 (8.8%) vs 79 (6.4%). (P=0.000). As more aneuploidies are observed among male blastocysts, the final number of available euploid blastocysts for embryo transfer was comparable between sexes (540 male/546 female), [OR (95%CI): 0.99 (0.87-1.11)].
Limitations, reasons for caution: This is a retrospective study. Only embryos at the blastocyst stage have been analyzed. Potential confounding factors such as sperm quality or the female age have not been analyzed. No data regarding the SR at birth have been analyzed in these study.
Wider implications of the findings: In our study, more male embryos develop to the blastocyst when compared to female ones. It can be hypothesized that female embryos can be more affected by an early arrest at cleavage stages. SR at birth would be expected to be similar as more aneuploidy is observed in male embryos.
Trial registration number: Not Applicable

P-562 PGT-M with de novo mutations : how to deal with it?

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Study question: How to technically deal with the PGT-M set-up in case of *de novo* mutations in female or male affected patients with dominant disease due to *de novo* mutations.
Summary answer: PGT-M was performed for three couples carrying *de novo* mutations using direct and linkage analysis on sperm or polar bodies to define haplotypes and phase.
What is known already: Couples with a *de novo* mutation in a gene causing AD disease, such as *FGFR3* (achondroplasia), *NF1* (neurofibromatosis) and *EXT2* (multiple exostosis) cannot undergo PGT-M via standard techniques like karyomapping, as the absence of affected relatives makes phasing impossible. However, linkage analysis combined with direct mutation analysis allows on haploid cells from the mutation carrier, such as sperm or polar bodies (PB), allows the correct association of a haplotype and the disease-causing mutation. Flanking informative STRs must be positioned at <1 Mb of the gene, in order to minimize the risk of recombination during meiosis.
Study design, size, duration: Couples underwent pre-test counselling with a geneticist and an IVF specialist. Pathogenic variants were identified and their absence from the couples' parents confirmed. Four to six informative STRs were identified. For males we analysed 20-50 isolated sperm to define the haplotypes and the phase, before starting with the stimulation cycle; for females, we needed to wait after the oocyte pick-up and the biopsy of PBs. Point mutations are identified by SNaPshot, deletions by multiplexed STS.
Participants/materials, setting, methods: The 3 couples in the study presented in IVF centres, requesting PGT-M for either male or female AD disease. They had genetic testing reports from other laboratories. For *FGFR3* and *NF1*, the described variants were confirmed. The patient with multiple exostosis came with a negative genetic result for *EXT1* and *EXT2* genes, but after diagnostic-quality NGS (Blueprint Genetics, Finland) we identified an *EXT2* deletion.

Diagnostic multiplex PCR was then performed on embryos or polar bodies.
Main results and the role of chance: The setup started with the confirmation of the mutations in the 3 couples and the confirmation of the *de novo* status. Four to six informative STRs were then identified for each couple. Multiplex PCR containing the STRs and the SNAPSHOT analysis for the point mutations was developed. To identify the phase and the disease-carrying haplotype in male carriers, we performed a multiplex PCR on 20-50 spermatozoa. In the female patient with *NF1*, the haplotype and the phase were determined on the polar bodies; the mutation was on her paternal allele, as predicted genetically.

Prior to PGT, we evaluated the robustness of each multiplex on 20 to 50 single leukocytes of the couple. Each couple had at least one embryo not carrying the risk haplotype, suitable for transfer. The couples with *NF1* and achondroplasia both delivered a healthy, unaffected baby. The pregnancy is ongoing in the couple with the *EXT2* variant.

PGT-M is now easily handled for standard situations, with semiautomated protocols that do not need extensive setups. *De novo* mutations however present a unique challenge, because of the impossibility in most cases of determining the phase of the disease-causing variant. We present a patient-centric approach with individualized protocols.
Limitations, reasons for caution: Allele drop-out could lead to misdiagnosis of the embryo. To avoid that, 6 flanking STRs (3 proximal and 3 distal) and genotyping of the variant should be performed. When possible, it is good practice to pre-define the different haplotypes with the parents of the patients.
Wider implications of the findings: The increasing number of laboratories offering off-the-shelf testing with NGS panels and semi-automated PGT can fulfil demand for routine situations. However in more complex cases, diagnostic-quality NGS and individualized PGT-M programmes are needed. These cases also remind us that PGT-M requires extensive multidisciplinarity to maximize the chance of successful outcome.
Trial registration number: not applicable

P-563 Assessing ovarian age: Could we use leukocyte telomere length as a surrogate marker of cumulus cells telomere content?

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Study question: Is leukocyte telomere length (LTL) correlated with cumulus cells telomere length (CCTL) in an age-heterogeneous women population?
Summary answer: LTL showed a positive correlation with CCTL in the studied population. Hence, its potential value as indicator of ovarian age would deserve further evaluation.
What is known already: Progressive telomere shortening has been related to ovarian aging and genomic instability during early development. A positive correlation between short telomere length of the first polar body and aneuploidy rate has been reported. CCTL has shown to be a biomarker of oocyte and embryo quality, but its assessment is impractical. LTL has been proposed as a surrogate of TL of follicular cells, but telomere lengthening through folliculogenesis could be controlled by different mechanisms. Thus, we aimed to determine if LTL in an age-heterogeneous population is correlated with CCTL and therefore considered an accurate surrogate for telomere length in the ovary.
Study design, size, duration: In this prospective non-interventional cohort study, 35 egg donors and 17 women undergoing Preimplantation Genetic Testing for Aneuploidy (PGT-A) treatment were included during sixteen months. Following controlled ovarian stimulation determined by treating physicians, oocyte retrieval was performed 36 hours after final maturation induction. Cumulus cells (CC) for telomere length (TL) measurement were obtained after the pick-up and oocyte stripping. A blood sample was collected through peripheral venous access for LTL measurement.
Participants/materials, setting, methods: Genomic DNA of CC and leukocytes from the 52 subjects was isolated. Average delta cycle threshold (ΔCt) was determined using a SYBR green quantitative real-time PCR protocol for relative TL. For normalization of measurements, a Taqman assay for the multicopy gene Alu was performed. ΔCtL and ΔCtCC were compared by a paired t-test analysis and the fold change was calculated. Additionally, the association between them and patient age was analyzed by a Pearson correlation test.
Main results and the role of chance: Mean participant's age was 29.94 ± 7.55 years and mean values for ΔCtL and ΔCtCC were 7.99 ± 0.53 and 7.46 ± 0.75, respectively. A positive significant correlation was found between age and ΔCt (ΔCtL: R2=0.71, p-value=5.18e-09; ΔCtCC: R2=0.47, p-value=0.00049). Since ΔCt values are inversely proportional to the amount of nucleic acids amplified and, therefore, to the telomere length, this correlation means that TL in both cell types decreases as women age. Additionally, ΔCtL was significantly higher than ΔCtCC (ΔCt fold change: 0.93, p-value=9e-07), meaning that CC showed significantly longer telomeres than leukocytes, thus supporting our previous published results in young egg donors. When analyzing the ΔCtL and ΔCtCC in these age-heterogeneous sample, a positive moderate and significant correlation was observed (R2=0.42, p-value=0.002). Thus, LTL could be suggested as a potential indicator of CCTL and therefore as a candidate for a biological marker of ovarian aging.
Limitations, reasons for caution: The sample size of this study was moderate and perhaps increasing the number of subjects might give additional strength to our findings. In addition, although relative telomere length allowed for adequate comparison between subjects, this method did not allow for absolute TL measurement.
Wider implications of the findings: While reproductive implications of LTL measurement need to be further studied, our results support the potential usefulness of LTL measurement as an indicator of CCTL and ovarian aging when analyzing an age-heterogeneous population. Further, our findings suggest that CC could possess different mechanisms to cope against telomere length shortening.
Trial registration number: Not applicable

P-564 Analysis of efficiency and outcomes of assisted reproductive technology (ART) programs after embryo transfer with a low-level of mosaicism

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Study question: How does embryo transfer with a low-level of mosaicism affect the success of ART programs, pregnancy, and live birth in comparison with euploid embryo transfer?
Summary answer: The transfer of mosaic embryos results in the delivery of a healthy baby however significantly decreases the outcome of ART programs and live birth rate.
What is known already: Present methods of preimplantation genetic testing of aneuploidy (PGT-A) allow detecting a mixture of euploid and aneuploid cells at the blastocyst stage with high accuracy. Such embryos are classified as mosaics with varying levels according to the guidelines of the International Society for Preimplantation Genetic Diagnosis (PGDIS). Numerous sources describe that number of mosaic embryos can vary from 4 to 22%. Several publications report that mosaic embryos can lead to successful pregnancies and healthy childbirth, but with a lower frequency and higher rates of pregnancy loss compared to euploid embryos. Nevertheless, the effect of mosaicism on ART outcomes remains controversial.
Study design, size, duration: It has been analyzed 2506 embryos from 648 patients undergoing the ART program with PGT-A at the Institute of Reproductive Medicine for 2018 - 2019. Embryos after PGT-A were classified as euploid, aneuploid, and having mosaicism of less than 40% as low level and more than 40% as high level following PGDIS guidelines. Patients of (group A) were transferred 467 single euploid embryos, and 43 patients (group B) underwent single low-level mosaic embryo transfer.
Participants/materials, setting, methods: The embryos on day 5 or 6 were graded by Gardner Scoring System. Approximately 5-10 TE cells were biopsied from good quality blastocysts and subsequently vitrified. PGT-A was performed utilizing an array comparative genomic hybridization (aCGH) (Agilent). The transfer of mosaic embryos was performed in the absence of an alternative, only after medical genetic counseling with a risk explanation and the subsequent signing of an informed agreement. Statistical tests processed by Pearson's chi-squared test.
Main results and the role of chance: Of all analyzed embryos, the proportion of euploid embryos was 48.6% (n = 1002), the total number of mosaics was 18.6% (n = 384) and aneuploid ones were 32.8% (n = 676). Depending on the level of mosaicism, the ratio between embryos with low-level mosaicism (≤40%) / high-level (≥40%) was 38.3% / 61.7%, respectively. According to the study, there was a significant decrease in the indicator of clinical pregnancy rate after embryo transfer with a low-level of mosaicism of 44.1% versus 63.2% transferred euploid embryo (p<0,01), however, despite an increase losses pregnancy in the group B (26.3%) there was no significant difference (p = 0.16) in comparison with the control group (15.4%). The live birth rate (LBR) significantly decreased (p<0.001) after the transfer of the mosaic embryo by 32.5%, while in the control group the indicator was 53.9%. In all cases, after the transfer of the mosaic embryo, healthy babies were born. There were 2 cases of high-level mosaic embryo transfer as a result of which pregnancy did not occur. According to the survey, about 70% of patients agree to replant mosaic embryos, 20% are ready to go to the new program, and 10% cannot make a decision.
Limitations, reasons for caution: The number of patients in group B was significantly lower than in group A. Not enough cases of embryo transfer with a high-level of mosaicism.
Wider implications of the findings: The current study might help to develop and to select a more appropriate strategy for transfer mosaic embryos. The next series of studies should focus on obstetric and neonatal outcome data from mosaic embryo transfer to gain a better understanding of the chromosomal and physiological health of children.
Trial registration number: not applicable

P-565 Does length really matter? The impact of lifestyle on granulosa cell telomeres length and IVF outcome

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Study question: Is the relative telomere length in granulosa cells associated with an abnormal metabolic profile of IVF patients and treatment outcome?
Summary answer: Longer telomeres and higher pregnancy rates have been observed in women with higher follicular fluid glucose levels.
What is known already: Telomeres may serve as a biomarker of cell senescence; their length varies depending on two factors: genetic predisposition and shortening processes related to cell division. In several studies, telomere shortening in granulosa cells has been shown to be correlated with ovarian aging. However, these findings are still limited in applicability, and more research is required to define the factors involved.
Study design, size, duration: Women undergoing IVF participated in a prospective cohort study between 2018 and 2019. Peripheral blood was obtained on the day of oocyte retrieval. Pooled samples of follicular fluid were collected for a comprehensive telomere length analysis in granulosa and cumulus cells by quantitative PCR (qPCR).
Participants/materials, setting, methods: DNA was extracted from granulosa cells and assessed for relative telomere length by monochrome multiplex quantitative PCR (qPCR). Telomere length was then analyzed relative to a single copy gene (36B4) to evaluate possible metabolic profile's impact on telomere length and treatment outcome. Hormonal profile, chemistry, and inflammation factors were analyzed in the follicular fluid and serum.
Main results and the role of chance: Out of forty-nine women recruited for the study, forty-one cases were eligible for a comprehensive analysis. Follicular fluid CRP and triglyceride levels in BMI-based analysis were significantly lower in women with BMI <25 compared to those with BMI >30 (0.5 (0.2-1.5) vs. 6.6 (3.6-10.6); $p < 0.0001$ and 21.04 ± 8.04 vs. 28.18 ± 8.97; $p = 0.011$, respectively). Interestingly, a significant correlation between the relative lengths of telomeres and pregnancy rate was observed ($p < 0.001$), with a higher pregnancy rate in women with longer telomeres (88%) than in women with shorter telomeres (38%). Relatively longer telomeres (0.96 (0.94-0.99) vs. 0.91 (0.82-0.95), $p = 0.02$) and higher levels of follicular fluid glucose (63±11.12 vs. 50.67±15.69, $p = 0.006$) were observed in those who conceived. No statistically significant differences were detected in the levels of the other hormones measured in the blood or follicular fluid on the day of oocyte retrieval or other clinical parameters, including follicle size and number, embryo quality, and the number of OHSS cases.
Limitations, reasons for caution: As a preliminary study, there were few participants. Further implications can be reached by increasing the sample size, but telomerase activity evaluation is required to confirm telomere length measurements without question. Whether short telomeres associate with infertility as a cause or consequence of BMI warrant more research.
Wider implications of the findings: The relative length of telomeres in granulosa cells at the time of oocyte retrieval may serve as a predictive biomarker for oocyte competence and subsequent pregnancy. Moreover, healthy lifestyle behavior is recommended.
Trial registration number: not applicable

P-566 Advanced paternal age can influence aneuploidy rate in egg donation cycles with poor sperm quality

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Study question: Could advanced paternal age influences the embryos aneuploidy rate in eggs donation cycles with poor sperm quality?
Summary answer: In case of severe male factors increased paternal age can affect embryos aneuploidy rate in egg donation cycles.
What is known already: While the impact of advanced maternal age on reproductive is well understood, the effect of paternal age on reproductive function is controversial. Many studies have shown that Advanced Paternal Age (APA) could impact on male fertility potential affecting testicular function and sperm quality. Moreover, APA also has been associated with increased epigenetics changes and DNA mutations. Increased paternal age could be associated with different types of disorders such as autism, schizopherenia and bipolar disorders. Egg donation cycles, controlling female variables, represent the ideal model for the study of the impact of paternal age on reproductive outcomes.
Study design, size, duration: We retrospectively analyzed 43 egg donation cycles (October 2014-January 2020) with ≥ 50% survival rate of vitrified/warmed oocyte. Only cycles with poor sperm quality were considered. Cycles were divided in two GROUPS: group-1 included male paternal age ≤ 45 while group-2 included male paternal age >45. Data, shown as avarage±SD, were analyzed with Chi square or Student-t test.
Participants/materials, setting, methods: Group-1 included 20 cycles and 219 oocytes, male age was 40,89 ±6.12; Group-2 included 17 cycles and 173 oocytes, male age was 51±6.06. Respectively, in Group 1 and in Group 2, donor age were 22.4±2.65 and 24.8±3.88 (NS). All oocytes were injected with abnormal sperm samples according to WHO 2010. Embryos were cultured in time-lapse system until blastocyst stage. Trophectoderm biopsy and PGT-A analysis were performed according to standardized laboratory protocols.
Main results and the role of chance: Oocytes survival rates in Group1 and 2 were 86% (188/219) and 90.7% (157/173) (NS), respectively. Fertilization rates in Group1 and -2 were 71.42 (135/189) and 73.45% (119/162) (NS), respectively. The total number of obtained embryos (transferred + frozen) were 81 and 801 in Group-1 and -2, respectively. The rates of obtained embryos per reiceved occytes were 37% (81/219) and 46.24% (80/173) in Group-1 and -2 ($p<0.7$), respectively. The PGT-A analysis showed 38.7% (31/80) and 31.17% (24/77) of euploid (NS) and 25% (20/80)and 42.85% (33/77) of aneuploid embryos ($P<0.05$) in Group-1 and -2, respectively. Mosaic embryos were 33.5% (26/80) and 27.27%(21/77), in Group-1 and -2, respectively. (NS). These results indicate that in presence of severe male factor, advanced paternal age could increase embryos aneuploidy rate raising incidence of chromosomal abnormalities.
Limitations, reasons for caution: Each donor was stimulated with different protocols according to her history and hormones levels. Nothing is known about which type of sperm parameters (semen amount, morphology or motility) have a major impact when focusing on the embryos genetic outcome.
Wider implications of the findings: To better known the effect of APA, it could be necessary identify embryos chromosomal abnormalities and the correlation with specific sperm parameters. Further studies should be done to confirm the APA effect in patients with severe male factors and define a cut-off male age where PGT-A should be recommended.
Trial registration number: not applicable

P-567 Re-establishment of fertilization competency of the oocyte via CRISPR/dCas9 epigenome edition technology

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Study question: Is it possible to increase the decreased levels of the sperm-oocyte binding protein, Juno, to restore the fertilization capacity of the oocyte, via the use of the CRISPR/dCas9 activation system?
Summary answer: JUNO domain (in the oocyte) suppressed with melamine was opened using the CRISPR/dCas9 system and sperm-oocyte binding and fertilization capability have been restored.
What is known already: Melamine is a chemical that is widely used in the manufacture of laminates, plastics, etc. Evidence reveals that long-term exposure to melamine could damage reproductive systems in mammals leading to infertility. Izumo1 is the only cell surface protein expressed on sperm that is known to be essential for sperm-egg interaction in vivo. It was in vitro shown that high-dose

feeding of melamine to female mice led to a significant decrease of JUNO on the plasma membrane of eggs. CRISPR/dCas9 system can provide the gene activation or repression to activate the target gene via Sam and Tet1 based systems.

Study design, size, duration: Six different gRNAs were designed for the transfection of oocytes. Six-week-old mice were fed with melamine (50mg/kg/day) for 2 weeks via gavage. Melamine gavage, water gavage, and control groups (n=15 /each group) were created. CRISPR activation plasmids (SAM) were given by piezo microinjection into the GV oocytes (n=100 oocytes/each group). Fertilization capacity was evaluated by sperm binding assay, qPCR, Western blotting, and IF staining. Two technical replicates were used in molecular studies.

Participants/materials, setting, methods: 293T cells were transfected (dCas9 SAM plasmids+gRNA) with Fugene. Mice randomly were assigned to 3 groups (n=15), as each was given orally a dose of 50mg/kg/d of melamine, only water or no water via gavage. Microinjection of plasmids was performed. Post-injection, oocytes were incubated until MII stage. For binding and fertilization evaluation, motile sperms were incubated with oocytes, and pronuclei were checked. Juno and IZUMO1 levels were evaluated by qPCR, Western blotting, and IF staining.

Main results and the role of chance: As the SAM system is more efficient compared to the Tet1 system when tested in 293t cells, the SAM system was used for mouse experiments. As a result of qPCR performed in oocytes collected at the end of gavage, it was observed that the JUNO expression levels were decreased by 40 folds in melamine fed mice ($p<0.05$). The decrease in the level of JUNO protein was demonstrated by IF stainings, and a decrease in the oocyte count along with abnormal uterine shapes was also observed in this group. IZUMO1 expression in motile sperms was demonstrated by qPCR before sperm binding assay and the position of the IZUMO 1 domain before the acrosome reaction was demonstrated by IF stainings. Sperm binding assay has demonstrated a 70% reduction in fertilization competency of melamine-treated oocytes ($p<0.05$). SAM plasmids and JUNO gRNA were given to oocytes by piezo injection. By sperm binding experiments conducted to evaluate fertilization capacities after microinjection, it was shown that the fertilization capacity was restored by 75% ($p<0.05$). Re-gaining of Juno expression in the oocytes was supported by a 60 fold increase in qPCR results. Recovery of JUNO protein expression in the oocytes was also demonstrated by IF stainings.

Limitations, reasons for caution: There is no known promoter region for the JUNO gene in the mouse. Therefore, we designed gRNAs targeting possible promoter regions. However, we have used two activation systems(SAM and Tet1) that are widely used to open a closed gene, but other activation systems (acetylation, etc.) can also be tried.

Wider implications of the findings: This study is valuable since: -it presents a possible cure for unsuccessful fertilization in related cases. -it possibly reveals melamine's unknown way of action. - it presents a new approach as a sperm-binding assay to be used in IVF clinics since Juno can also be expressed in somatic cell lines.

Trial registration number: non-clinical trials

P-568 Homozygous Pathogenic Variants in ACTL9 Cause Fertilization Failure and Male Infertility in Human and Mouse

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Study question: What are the other male factors that cause total fertilization failure (TFF) excepting for variants in *PLCZ1*?

Summary answer: Homozygous variants in *ACTL9* (actin like 9) cause abnormal localization of PLCζ in a loosened perinuclear theca (PT) structure and leads to TFF.

What is known already: In previous studies, investigators have reported that the female factors in TFF after intracytoplasmic sperm injection (ICSI) include pathogenic variants in *WEE2*, *TLE6*, and *TUBB8*, whereas for male factors, pathogenic variants in *PLCZ1* were reported to be the primary cause of TFF, which account for approximately 30% of couples with male factors in TFF excluding globozoospermia. Most recently, it was reported that pathogenic variants in *ACTL7A* led to reduced expression and abnormal localization of PLCζ, thereby identifying this genetic variant as a potential cause of TFF.

Study design, size, duration: Fifty-four infertile couples with TFF or poor fertilization (fertilization rate of <20%) at the Reproductive and Genetic Hospital of CITIC-Xiangya during January 2014 to June 2020 were recruited into this study.

Participants/materials, setting, methods: Male factors were identified in (MOAT). WES analysis was used to analyze the genetic factors of individuals with male factors. Sperm morphological study was conducted by H&E staining and TEM. Immunostaining of PLCζ was used to analyze the status of sperm-borne activation factor. A knock-in mouse model was generated by CRISPER-Cas9 technology. Sperm from homozygous *Actl9* variant mice were analyzed by TEM and ICSI. ICSI with AOA was performed in couples with *ACTL9* variants.

Main results and the role of chance: A total of 54 couples with TFF or poor fertilization were screened, with 21 couples determined to have a male infertility factor by MOAT. Whole-exome sequencing of these 21 male individuals identified three homozygous pathogenic variants in *ACTL9* in three individuals. *ACTL9* variations led to abnormal ultrastructure of the PT, with PLCζ absent in the head and present in the neck of the mutant sperm, which contributed to failed normal calcium oscillations in oocytes and subsequent TFF. The key roles of *ACTL9* in the PT structure and TFF after ICSI were further confirmed in *Actl9*-mutated mouse model. Furthermore, assisted oocyte activation by calcium ionophore exposure successfully overcame TFF and achieved live births in a couple with an *ACTL9* variant.

Limitations, reasons for caution: The mechanism of how ACTL9 regulate PLCζ remains unknown.

Wider implications of the findings: It provided a genetic marker and a therapeutic option for individuals who have undergone ICSI without successful fertilization.

Trial registration number: not applioable

P-569 Woman with CYP19A1 TC/CC genotype have increased susceptibility to infertility development, independently of the cause

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Study question: Is TC/CC genotype of codon 39 at CYP19A1 gene associated with the development of female infertility?

Summary answer: Yes, CYP19A1 codon 39 TC/CC genotype is associated with increased susceptibility to infertility development in women, regardless of associated cause.

What is known already: Aromatase protein is responsible for the aromatization of androgens into estrogens. This protein that catalyzes the final step in biosynthesis of estrogens is encoded by the gene CYP19A1. The CYP19A1 gene is located on chromosome 15q21.1. It is a member of the cytochrome P450 superfamily which are monooxygenases that catalyze many reactions involved in steroidogenesis. TC/CC genotype of codon 39 at CYP19A1 gene results in an increase of aromatase activity and thus affect the hormone levels which can lead to the development of various diseases, including infertility.

Study design, size, duration: A case-control study was designed to investigate the association of CYP19A1 gene polymorphism with female infertility. Case subjects, 201 women with infertility established as women under 39 years of age, that failed to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse. 161 fertile female controls, with no previous history of infertility, no previous history of gynecological pathologies compatible with infertility, and no history of IVF treatments, were selected.

Participants/materials, setting, methods: Blood was collected by venous puncture and genomic DNA was extracted. CYP19A1 genotyping was performed by polymerase chain reaction-based methods with confronting two-pair primers. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated by unconditional logistic regression.

Main results and the role of chance: Significant statistical association of the TC/CC genotype combined with endometriosis risk was found, with

reference to TT genotype (OR 4.554; 95% CI 2.209-9.386; p<0.001). We also found an increased risk of developing polycystic ovary syndrome (PCOS) associated with TC/CC genotype (OR 5.317; 95% CI 2.767-10.215; p<0.001). We also observed an increased prevalence of premature ovarian failure associated with TC/CC genotype (OR 3.376; 95% CI 1.672-6.815; p=0.001) and verified an increased prevalence of tubal pathology in carriers of TC/CC genotype (OR 3.231; 95% CI 1.653-6.314; p=0.001). Finally, a strong association of TC/CC genotype with female infertility, regardless of the cause was found (OR 4.232; 95% CI 2.710-6.609; p<0.001). In conclusion, TC/CC genotype is associated with increased susceptibility to infertility development in women.

Limitations, reasons for caution: The sample size may eventually be considered small, despite the strong significance found.

Wider implications of the findings: There are not many studies in this area and the few existing exhibit disparate results. The association of TC/CC genotype with endometriosis was observed in a few studies, but some disagree. This difference could be attributed to the notable heterogeneity across the different studies.

Trial registration number: Not applicable

P-570 Embryos originating from oocytes with smooth endoplasmic reticulum clusters have a lower euploidy rate via PGT-A testing using next-generation sequencing

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Study question: Does the presence of smooth endoplasmic reticulum clusters (sERCs) in oocytes affect the human embryo ploidy?

Summary answer: The euploidy rate of embryos originating from sERCs + oocytes is lower

What is known already: While an expert panel strongly recommended that sERCs+ oocytes should not be inseminated, some normal healthy babies derived from sERCs+ oocytes have been reported. In previous studies have shown that declined fertilization rate and lower proportions of good quality embryos are found in oocytes showing sERCs. The updating findings of the molecular status of sERC+ oocytes elucidated the sERCs+ oocytes may have impaired chromosomal segregation ability. However, no study reveals the relation between sERCs and embryo ploidy.

Study design, size, duration: A retrospective study enrolled 129 preimplantation genetic testing (PGT) cycles from January 2017 to March 2020 at Chang Gung Memorial Hospital, Lonkou.

Participants/materials, setting, methods: ICSI fertilization rate, Day5 usable blastocyst rate (D5UBR), total usable blastocyst rate (TUBR), euploidy rate, mosaic rate, and aneuploidy rate are investigated between embryo originating from sERCs+ and sERCs- oocytes.

Main results and the role of chance: Although higher TBUR in blastocyst derived from sERCs+ oocytes than sERCs- group (73.7% vs. 62.5%) but accompanied lower euploidy rate (7% vs. 29%) and higher aneuploid rate (79% vs. 54%).

Limitations, reasons for caution: Limited sample size, need a large-scale study to confirm the conclusion. The live-birth rate per embryo transfer cycle was not included for analysis. As we did not perform polar body analysis, we cannot state for sure that embryonic aneuploidy was related to the oocyte.

Wider implications of the findings: This study demonstrates that embryos originating from sERCs+ oocytes have a lower euploidy rate.

Trial registration number: CMRPG3H0751

P-571 Preimplantation Genetic Testing for Aneuploidies (PGT-A) and pregnancy outcome

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Study question: Frozen embryo transfer (FET) of euploid blastocysts in hormone replacement therapy (HRT) or natural cycle (NC): are there differences in obstetric, fetal and neonatal outcomes?

Summary answer: Pregnancy complications, neonatal outcomes and fetal abnormalities are not increased after FET with PGT-A in singleton pregnancies.

What is known already: Since its introduction, PGT has been widely used in ART centers for preventing chromosomal and monogenic diseases. Despite its increased use, there are scarce and conflicting data about adverse pregnancy, fetal and neonatal outcomes. In one published study, the risk of preeclampsia and placenta previa was increased when PGT pregnancies were compared with non-PGT, while the incidence of gestational diabetes mellitus (GDM), preterm delivery, fetal defects and NICU (Neonatal Intensive Care Unit) admission were similar. According to other data, the rate of caesarean section in PGT pregnancies was high - around 80% in singletons.

Study design, size, duration: An observational, retrospective study was conducted between March 2015 and November 2019 in patients with singleton pregnancies after ART with PGT-A/FET/HRT and NC.

A total number of 353 patients from two fertility centers (ART Fertility Clinics Dubai and Abu Dhabi, UAE), were included. They were divided into two groups according to the endometrial preparation for FET: group A: HRT (n=225) and group B: NC (n=128).

Participants/materials, setting, methods: Patients with primary / secondary infertility and at least one transferable euploid blastocyst after trophectoderm biopsy, achieving an ongoing singleton pregnancy after FET were included. Endometrial preparation for FET was either performed in a NC or an HRT cycle. For this study, the following pregnancy outcomes were recorded: GDM, preeclampsia and hypertension, obstetric cholestasis, placental abnormalities, mode of delivery, preterm delivery, gestational age at delivery, birth weight, fetal abnormalities and admission to NICU.

Main results and the role of chance: There were no statistically significant differences in maternal and demographic characteristics of the studied groups. The mean maternal age was 34.05(20-45) and 34.26(23-47) years for group A and B respectively. The mean BMI was 28.31kg/m² (17.93-43.76) versus 27.93 (17.32-43.18). The ratio of nulliparous versus multiparous patients was 1:1 for both groups. Majority of the patients in both groups were of Arab ethnicity. The number of patients recorded as smokers was low and comparable in the groups. The mean gestational age at the time of delivery was comparable: 37.64 gestational weeks (24-41) versus 37.76 (26-41). The Caesarean section rate was around 50% for both groups. The rate of preterm delivery was comparable in both groups (16.9% and 18.8% for group A and B respectively). There was no detectable difference in the distribution of the birth weight in both groups with a median weight of 3000 grams of which 13.6% were low birth weight. In the studied groups, 30.5% had pregnancy complications with no observed statistically significant differences when the groups were compared. There was no increased incidence of fetal abnormalities. Admission to NICU was comparable and was related to prematurity.

Limitations, reasons for caution: The limitations of the study are the retrospective design and the small number of participants.

Wider implications of the findings: In patients with FET of an euploid embryo after PGT-A, the type of FET treatment preparation (HRT or NC) has no significant effect on pregnancy complications, birth weight and fetal abnormalities. The findings of the present study could be used to improve prenatal counselling for women undergoing ART with PGT-A.

Trial registration number: Not applicable

P-572 Purifying selection for aneuploidy cells in mosaicism embryo at post-implantation stage

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Study question: Why low ratio mosaicism embryos develop to normal karyotype babies?
Summary answer: Our in vitro implantation assay clarified purifying selection for aneuploid cells in post implantation embryos.
What is known already: There are some reports about healthy live birth after transfer of mosaic embryos, which was reported for the first time from Italy in 2015. It is also reported that the abnormal cell is screened with the mouse in the embryo development, and only a normal cell contributes to the development. But it has not been examined in human.
Study design, size, duration: To clarify the change of aneuploid cells and mitochondrial activity in human embryo, we biopsied several parts from one blastocyst and examined karyotype. After in vitro implantation assay for biopsied embryos, we compared the karyotype of biopsy sample with that of cultured cell mass.
Participants/materials, setting, methods: Under the ethical review of Yokohama City University and informed consent with patients, we collected human surplus blastocysts those are donated after successful clinical treatment or discarded because of poor development grade. We biopsied multiple parts from one blastocyst and cultured the biopsied embryos, and extracted whole DNA from the biopsy samples and cultured embryos. Karyotyping by next generation sequencing were performed.
Main results and the role of chance: We analyzed 34 samples from 11 embryos, including 25 biopsy sample from 11 embryos and 9 cell mass from 7 cultured embryos. In the karyotype tracking results, even though biopsy sample analysis before the culture were uniformed aneuploid or chromosome mosaic, the developing embryo cell mass had normal karyotype. In one embryo as an example, among the three biopsied extra trophectoderm samples from that, two of them were mosaic, and one of them had uniformed chromosome 21 trisomy and chromosome 16 mosaic monosomy. But the embryo formed multiple cell mass in implantation assay. We examined karyotype of three cell mass, and the result from all were normal karyotype. We suggested that the chromosome aberration cells were screened in the human embryo development, and when the function was not carried out the embryo stopped the development.
Limitations, reasons for caution: Because of small number of samples available, we need more samples for a more accurate evaluation. Furthermore, we cannot evaluate the absolute mechanism that cells with chromosome aberration decreases.
Wider implications of the findings: Conventional PGT-A techniques are based on uniformed embryos developing hypothesized past time. As showed in some clinical reports, PGT-A can reduce of spontaneous abortion and chance of embryo transfer. Thinking about aneuploid cell purifying system in embryo development, effectiveness of PGT-A should be more questionable for infertility treatment.
Trial registration number: A200326004

P-573 PGT-M for two or more disease carrier patients diagnosed after whole exome sequencing

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Study question: Can whole exome sequencing (WES) before PGT-M identify previously unknown mutations for consanguineous couples having an increased risk of carrying more than one genetic disease?
Summary answer: WES has been successfully applied in combination with PGT-M by identifying new pathogenic mutations in addition to known gene mutations, extending the scope of PGT-M.
What is known already: Most couples ignore their risk of being a carrier of an inherited genetic disease until they have an affected child. Rare, atypical, and undiagnosed autosomal-recessive disorders frequently occur in the offspring of consanguineous couples. Routine single gene diagnostic tests fail to detect any possible gene defects other than the clinically apparent one. Prospective WES or genetic carrier screening testing of consanguineous couples could identify couples who both are carriers of autosomal recessive diseases and thus encourage them to make informed reproductive decisions. Screening tests using NGS technology simultaneously sequence exons and exon-intron boundaries to determine disease carrier status.
Study design, size, duration: Between January 2017 and October 2020, a total of 206 PGT-M couples applied to Istanbul Memorial Hospital ART Center. Of these couples, multigene PGT-M workups were carried for twelve couples who were carriers of more than one inherited disease. Eight couples were found to be carriers for two different diseases and four couples were carrying three diseases. All biopsies were performed at the blastocyst stage.
Participants/materials, setting, methods: For the 12 couples with multigene PGT-M workups the average female age was 31.0 ± 6.2. Nine of them initiated an ART cycle and the mean number of cumulus-oocyte complexes, metaphaseII oocytes, biopsied blastocysts and transferrable PGT-M embryos were 15 ± 6.9, 13.3 ± 6.3, 5.9 ± 2.0 and 2.9 ± 1.9, respectively. PGT-A was routinely performed for all couples with transferrable PGT-M tested embryos except one couple who refused PGT-A.
Main results and the role of chance: A total of 28 different gene workups were completed for 26 genes. The inheritance mode of the 26 conditions was as follows: 20 autosomal recessive, four autosomal dominant and two X-linked recessive. Out of 12 couples, 9 of them initiated an ART cycle and transferrable embryos were found after PGT-M followed by PGT-A. Eight women had frozen embryo transfers resulting in five healthy babies (3 singletons and 1 twin), two pregnancies still ongoing and one biochemical miscarriage at the time of data collection. The couple who declined PGT-A testing prior to their frozen embryo transfer had anegative bhCG test. Three couples completed their workups but postponed their ART and PGT-M cycle due to Covid-19 pandemic.
Limitations, reasons for caution: The probability of finding at least one transferrable embryo after PGT-M is influenced by the inheritance mode of the disease. Late-onset diseases presumed to be caused by variants of unknown significance and polygenic diseases that are possibly influenced by environmental factors were not included in this study.
Wider implications of the findings: With decreasing costs and improved availability of WES and genetic carrier screening panels, couples, especially consanguineous couples, who were previously shown to have one inherited disease may be offered to be screened for additional undiagnosed inherited diseases that may pose a threat for their offspring.
Trial registration number: Not applicable

P-574 Examination of inter centre variation in PGT-A "no result rate" and efficacy of rebiopsy - Analysis of 22,833 samples 2015-2019

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Study question: Are there significant differences in PGT-A "no result" rates and clinical outcomes following rebiopsy between ART clinics, and do rebiopsied embryos perform better than transferring with no result?
Summary answer: There is significant differences between clinics in terms of "no result rate" in PGT-A and utilisation of rebiopsy. What is known already: With any testing platform used in PGT-A, there is always a chance that a sample will not yield a result and rebiopsy may be considered to ascertain an embryos cytogenetic status. Studies have demonstrated rebiopsy yields results and adds to embryos genetically suitable for transfer. Clinical outcome data, however, remains scarce, leading to difficulty for clinics in benchmarking their performance when rebiopsied embryos are transferred.
Study design, size, duration: A retrospective analysis was performed of trophectoderm samples submitted for PGT-A via NGS over a 5yr period, 2015-2019. The no result (NR) rate was calculated per year and per clinic. Clinics were contacted for follow up data on NR embryos in terms of usage and clinical outcomes. Clinical outcomes from rebiopsied embryos were compared with those transferred as NR without rebiopsy.
Participants/materials, setting, methods: Data was collected on 22833 trophectoderm samples, submitted by 30 IVF laboratories. NR rate was analysed by year and by clinic. Clinics were asked if NR embryos had undergone rebiopsy, and if so if they had survived warming and rebiopsy. Clinics were asked if embryos selected for transfer had survived (re)warming, and to provide clinical

follow-up including hCG test, clinical pregnancies, miscarriage and livebirth. The two tailed Fishers exact test was used for statistical analysis.
Main results and the role of chance: There was a wide range in sample numbers submitted by clinics over the time period, ranging from 9 samples through to 2633. In tclinics submitting over 500 samples the NR rate ranged from 0.6% to 7.4%, and in the those submitting 100-499 samples it ranged from 1.1% to 5.8%. Both these differences proved to be statistically significant ($p<0.05$) between the best and worst performing clinics, and shows that a gap in performance exists between clinics. Less than 50% of NR embryos underwent rebiopsy. While the majority of embryos undergoing rebiopsy yielded a result (92.3%) and 31.4% of these were euploid or mosaic, almost half still remain in storage. The rate of livebirth/ongoing implantation in the rebiopsy group is 35.5% and 17.1% in the non rebiopsy group, illustrating a non significant trend towards a higher chance of implantation and livebirth in the rebiopsy group. Of 58 patients undergoing rebiopsy without any euploids in their initial cycle, 18 had a euploid embryo identified for future use. The additional manipulations involved in rebiopsy do not impact on survival at warming for transfer, but clinical outcomes in rebiopsied embryos appear poorer than those where a result was generated at first biopsy.
Limitations, reasons for caution: Despite starting with 22833 samples, 1115 of which were classified as NR, there were only 31 rebiopsied and 42 NR embryos transferred. It was therefore not possible to analyse transfer data by clinic or by embryo quality.
Wider implications of the findings: Rebiopsy yields genetic results and embryos suitable for patient use, including for patients who produced no other euploid/mosaic embryos in their cycle. However, it is not offered/performed in many cases. Clinical outcome data must continue to be compiled and analysed to confirm performance exceeds transfer of NR embryos.
Trial registration number: not applicable

P-575 Patients with recurrent implantation failures (RIF): chromosome abnormalities in the resulting embryos

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Study question: Do RIF patients have the preimplantation genetic testing for aneuploidy (PGT-A) overcome their infertility condition?
Summary answer: PGT-A positively impact on implantation rate in RIF patients
What is known already: The most common definition of RIF is failure to achieve a pregnancy after three consecutive transfers of good quality embryos. This term possibly represents a heterogeneous category of infertile couples as the causes of repeated failures can be diverse. Especially intriguing is the case of patients with an age lower than 39 years for which the oocyte quality is expected not to be compromised by the well known age effect on female fertility. The chromosome analysis of the resulting embryos has been proposed as a valid method to improve implantation in the great majority of RIF patients
Study design, size, duration: This retrospective study included 49 patients with at least three previous consecutive implantation failures, which underwent PGT-A from January 2016 to April 2020. Both partners had a normal karyotype. Only patients with a female age below 39 years were included, who presented with a normal uterine cavity. Couples with a severe male factor were excluded. Single frozen blastocysts were transferred according to chromosomal results
Participants/materials, setting, methods: Maternal age was 35.5 ± 3.1 years. All blastocysts were vitrified after trophectoderm biopsy. Whole genome amplification and array comparative genomic hybridization were performed on biopsies. Only euploid embryos were transferred. The primary outcome was the live-birth delivery rate after the first transfer
Main results and the role of chance: Before starting a PGT-A cycle, these patients underwent 213 embryo transfers with 251 embryos replaced. A total of 264 blastocysts were analyzed, 140 of which were aneuploid (53%). Monosomy or trisomy was reported in 67 of the diagnosed samples (67/140, 48%) whereas the remaining 73 carried complex aneuploidies (73/140, 52%). The remaining 124 blastocysts (47%) were diagnosed as euploid. All patients performed an embryo transfer resulting in 28 clinical pregnancies (57%). There were 5 spontaneous abortions and the live-birth delivery rate per patient was 47%
Limitations, reasons for caution: This study suffers from the weakness related to retrospectivity. In addition, as euploid embryos are still cryopreserved, the delivery rate could change at completion of the cycles
Wider implications of the findings: A RIF condition can be attributed, at least in a good proportion of cases, to the generation of high percentages of aneuploid embryos. In this case, the transfer of euploid blastocysts has high chances to classify this category of RIF patients has having an embryonic cause of infertilit.
Trial registration number: Not applicable

P-576 Preimplantation genetic testing for consanguineous couple carrying an identical reciprocal translocation: clinical, ethical considerations and quandaries

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Study question: Which ethical and clinical aspects should be considered for preimplantation genetic testing PGT-SR strategy management for a consanguineous couple carrying the same reciprocal translocation?
Summary answer: PGT-SR management required specific probe designs to distinguish chromosomal patterns of balanced embryos, leading to complex transfer choices that required an adapted genetic counseling.
What is known already: Reciprocal translocation is a classic case in PGT-SR management, since all balanced embryos are transferable without distinguish between normal and balanced embryos. In accordance with the several recommendations, professionals calculated the reproductive risk related to the abnormalities and established an appropriate genetic counseling. However, an extreme case, such as the same reciprocal translocation carried by both members of a couple complicates PGT-SR management at all levels. Mainly, the genetics counseling around balanced embryo transfers. To date, only one study has reported a similar case, however, genetic counseling and the choice of embryos to be transferred have been poorly documented
Study design, size, duration: This study reports an extremely rare case of a couple (26-year-old woman and 29-year-old man) who was referred to our PGT center of the Montpellier University Hospital after 4 spontaneous miscarriages. The couple, first degree cousins in whom both partners are carrying the same reciprocal translocation 46,XX/XY,t(3;18) t(q26.1;q12.1). The patients were informed of the investigations and gave their consent before participation in the study.
Participants/materials, setting, methods: Peripheral blood of each member was investigated by FISH to caracterize chromosomal breakpoints. Secondly, a theoretical estimation of different segregation products to find a normal or balanced embryos were performed considering the extreme complexity of the case. Finally, an adapted PGT-SR probe strategy was conceived and proposed to a couple. Choices of balanced embryos to transfer were detailed to ensure that the patient is aware of risks and potential benefits.
Main results and the role of chance: In this particular case where both members of the couple are carrying the same reciprocal translocation, the chance of finding a normal or balanced embryo was further lowered 2% (4/196). It is estimated that the couple would produce 1 normal embryo and 15 balanced embryos. Diagnostic was possible on 16 biopsied embryos on day 3. Probe signal interpretations revealed four balanced embryos. Two embryos were proposed for a transferred on day 4. These balanced embryos had a different probe patterns, the first balanced embryo was normal and the second balanced embryo resulting from an adjacent-1 segregation mode presented an uniparental disomy (UPD).
Limitations, reasons for caution: ESHRE recommendations were established for common chromosomal rearrangements. In specific cases, limitations are strongly related to the complexity of the human genome. In this study, the choice of the embryos to be transferred depended entirely to our knowledge of phenotypic consequences of a homozygous gene alterations involved in chromosomal breakpoints.
Wider implications of the findings: Professionals were confronted with requests to transfer balanced embryos with a partial/complete UPD or a balanced double translocation homozygote to improve the transfer rate from 3/196 of balanced combinations to 16/196. Dilemma between risks and benefits were considered for counseling to ensure an informed decision-making by patients.
Trial registration number: NA

P-577 Impaired double strand DNA repair in isolated Primary Ovarian Insufficiency with homozygous nonsense mutation of SPIDR

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Study question: To identify the etiology of isolated Primary Ovarian Insufficiency (POI) in a patient from an Indian consanguineous family.
Summary answer: A homozygous nonsense mutation of SPIDR in the patient yielded chromosomal instability: first evidence of a role of this gene in DNA repair.
What is known already: POI, affecting 1% of women under 40, is a public health problem. To date ~ 70% of cases remain idiopathic. The leap due to exome sequencing, led to the identification of ~ 80 genes, often in single or few cases. SPIDR was recently identified as a scaffolding protein connecting RAD51, a central player in homologous recombination, to BLM, a helicase implicated in the integrity of the genome. But its precise role is still unknown. A SPIDR mutation was previously associated with POI. However, contradictory conclusions were reported on the mechanism of SPIDR action and on its pathogenic role in POI.
Study design, size, duration: Prospective genetic study of a cohort of 150 pateints with POI worldwide using a custom-made targeted next generation sequencing (NGS) panel comprising 60 known POI-causing genes. A single patient was found mutated in *SPIDR*. Cytogenetic studies were performed to analyse the consequences of the mutation on DNA repair and sister chromatide exchanges (SCE).
Participants/materials, setting, methods: The patient with *SPIDR* mutation had POI with primary amenorrhea, delayed puberty and streaks ovaries. She was born to consanguineous Indian parents. No other mutation was detected in our cohort of 150 patients with POI. Targeted NGS was performed in the proposita. Familial segregation was performed by Sanger sequencing. Mitomycin C (MMC)-induced chromosomal breakages were studied and a sister chromatid exchange (SCE) assay was performed in patient's peripheral lymphocytes.
Main results and the role of chance: We identified a novel homozygous nonsense mutation in the exon 7 of *SPIDR (KIAA0146)* c.814C>T, R272*, predicted to yield either a truncated protein, or a non-sense-mediated mRNA decay. The patient's cells display increased chromosomal fragility with high MMC-induced chromosomal breaks when compared to a control. Remarkably, there was no increased SCE. In the previous report of a SPIDR mutation in POI, no cytogenetic studies were performed, and contradictory results were obtained on a homologous recombination test between the two sisters, either enhanced or reduced. In conclusion, we show here that inactivation of *SPIDR* results in a defect of double strand DNA damage repair, similar to alteration of the RAD51 pathway. There was no increased SCE, the hallmark of the BLM pathway. This observation has major consequences for this patient's care : indeed mutations of DNA-repair genes may also yield to tumors/cancers. A long follow-up of the patient is needed in a multidisciplinary team to detect possible comorbidities. Indeed, even in the absence of somatic symptomatology, the patient has enhanced chromosomal instability highlighted by cytogenetic studies, that may yield tumor-predisposition.
Limitations, reasons for caution: No other mutation of SPIDR in the replication cohort of 150 POI patients. SPIDR mutation are thus very rare world-wide.
Wider implications of the findings: This is the first evidence of chromosomal instability associated with *SPIDR* defect, providing strong evidence for a role of SPIDR in double strand DNA damage repair in humans and for its causal role in POI. Our study improves the knowledge on SPIDR function and confirms its involvement in POI worldwide.
Trial registration number: not applicable

P-578 Human pappiloma virus 16,18 genome methylation patterns in subfertile women

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Study question: A comparison between L1 gene and LCR region methylation status of HPV16 and HPV18 viruses in subfertile women, investigating HPV methylation pattern in cervical cancer and asymptomatic HPV infection.
Summary answer: CpG methylation was more frequent in L1 gene compared to LCR in both HPV types. Methylation levels were associated with the grade of cervical dysplasia.
What is known already: HPV infection is a common sexually transmitted disease, related to genital warts and cancer. DNA methylation as a dynamic and strictly controlled process can be involved in numerous cellular processes, cell differentiation, gene expression regulation and genome reprogramming. Human pappiloma virus genome epigenetic alterations may play a key role in HPV life cycle as well as in the oncogenic process in general. However, whether the prevalence of high risk HPV is correlated with female infertility, has yet to be elucidated.
Study design, size, duration: From January 2015 to December 2019, about 2505 infertile couples were referred to the Human Reproduction Unit of Ioannina University Hospital. A total of 212 clinical and laboratory data from female partners were included in the study.
Participants/materials, setting, methods: Cervical smears were studied for HPV DNA methylation. CpG methylation was compared among L1 gene and LCR region in both HPV types. A bisulfite modification assay followed by DNA amplification and sequencing was performed to analyse HPV16 and HPV18 genome.
Main results and the role of chance: In HPV16 types, L1 gene and promoter region indicated high methylation levels in cervical cancer cases. LCR regions methylation levels ranged from 0,5% to 24,2% in asymptomatic HPV16 infection or cervical intraepithelial neoplasia and cervical cancer, respectively. As for L1 gene, the differences between asymptomatic HPV16 infection and cervical cancer cases were statistically significant (P=0.003). In HPV18 types, L1 gene was methylated in cervical intraepithelial neoplasia and cervical cancer cases. Promoter region methylation levels were high in cervical cancer cases while LCR region methylation levels were low.
Limitations, reasons for caution: Main limitation is the relatively small size of the collected samples.
Wider implications of the findings: HPV genome investigation, as for methylation status, may lead to better understanding and earlier diagnostics of cervical pathology in infertile population. These observations point out the importance of fertility preservation in women at high risk for cervical neoplasia.
Trial registration number: Not applicable

P-579 Pregnancies following preimplantation Genetic Testing have an increased risk for post-partum complications

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Study question: Do preimplantation genetic testing (PGT) pregnancies have higher pregnancy and delivery complications compared to naturally conceived (NC) pregnancies?
Summary answer: PGT pregnancies do not have increase pregnancy complications but do have increased post-partum complications.
What is known already: There is limited data about the outcome of PGT cycles regarding pregnancy complications.

Previous reports show that PGT pregnancies are similar to NC pregnancies regarding birth weight and preterm delivery rate.

Patients performing PGT are less likely to have infertility as a background problem, and therefore it is important to evaluate pregnancy complications in this specific population.
Study design, size, duration: A retrospective cohort study, between 2008-2020 in Shaare Zedek Medical center (SZMC). Demographic, background variables, treatment cycle information, and delivery data were collected from computerized hospital databases and patient files.

Participants/materials, setting, methods: All patients aged 18-45 that conceived following PGT treatment in the IVF unit and gave birth in SZMC were included in the study.
We used two control groups: (1) women with spontaneous pregnancies (SP) who gave birth in SZMC. We used four "neighborhood control" for each PGT patient (two women delivered before and two after the case delivery). (2) pregnancies following ICSI with four neighborhood control for each.
Main results and the role of chance: 135 deliveries following PGT, 924 ICSI, and 4199 NC. Demographic variables were similar except PGT, and ICSI women were slightly older (30.93 ±4.33 PGT, 31.70±4.98 ICSI, 28.75±5.69 spontaneous, p<0.01). PGT pregnancies had similar rates of placental complications (hypertensive disorder, preeclampsia (PET), placental abruption) as NC (p=0.8), while ICSI pregnancies had significantly higher rates of gestational hypertension (p<0.01) and abruption (p=0.05). We found a higher rate of preterm delivery <37 weeks in both PGT and ICSI pregnancies (23.7%, 22.7%, 12.1%, p<0.01 for PGT, ICSI, NC respectively), but only in ICSI was preterm delivery < 34 weeks increased (2.2% vs. 2.1%, p=0.9, for PGT and NC, 4.3% for ICSI p<0.01). Post-partum complications were more prevalent in both PGT and ICSI: longer third stage of labor (13.27±12.81, 12.58±10.08, 10.58±8.14, p<0.05), manual lysis of placenta (6.7%, 2.3%, 1.4% p<0.05), post-partum hemorrhage (PPH) (5.9%, 4.2%, 2.5% p=0.02) and need for blood products (3.7%, 4.5%, 1.3% p=0.02) for PGT, ICSI, NC respectively. The aOR for composite post-partum complications (PPH, hemoglobin drop>3 gram, revision or lysis) was 2.4, 95%CI [1.6-3.7].

We did not find any difference between fresh and frozen cycles in either placental complications, preterm delivery, or post-partum complications in the PGT group.
Limitations, reasons for caution: A single-center retrospective study. Included only pregnancies both conceived and delivered in SZMC.
Wider implications of the findings: Physicians should be aware of PGT pregnancies as risk factors for post-partum placental complications and handle the third stage of the delivery with caution.
Trial registration number: 0351-18-SZMC

P-580 Levels of the constitutive heterochromatin (cHC) mark H3K9me3 in human sperm in relation to IVF outcome and preimplantation embryo development

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Study question: Can we quantify the variation in epigenetic signature of constitutive heterochromatin (cHC) in human sperm using Western blot analysis and correlate this with pregnancy outcome after IVF?
Summary answer: Variation in the quantified H3K9me3 to H3 showed an association with embryo morphokinetics and a H3k9me3/H3 ratio <0,5 is negatively associated with pregnancy outcome.
What is known already: In human sperm, 5-15% of the DNA remains associated to histones. We previously showed that human mature spermatozoa still exhibit the canonical marks H3K9me3 and H4K20me3 on cHC regions and transmit these nucleosomes to the embryo after fertilization. Additionally, other groups reported an increase in the nucleosome/protamine ratio when sperm from male factor subfertility patients were compared with sperm from fertile men, indicating that incomplete chromatin remodelling during spermatid elongation may affect fertility. Thus, variations in sperm chromatin occur during human spermatogenesis that go undetected by current sperm quality tests and may have clinical significance for IVF treatment outcomes.
Study design, size, duration: An observational study of patient couples (n=53) undergoing IVF treatment at the Erasmus MC, Rotterdam, between 2017 and 2018. Inclusion criteria were normospermia according to the WHO criteria, at least 4 oocytes harvested after ovum pickup and an embryo-transfer. After routine insemination, resulting embryos from 38 cycles were cultured in the EmbryoScopeTM and developmental timings up to the 8-cell stage were recorded. The primary outcome measure was ongoing pregnancy (ultrasound at 12 weeks of gestation).
Participants/materials, setting, methods: Surplus sperm samples from 53 IVF cycles were lysed at a concentration of 200x106 spermatozoa/ml in RIPA buffer. Lysates were loaded and separated on SDS-PAGE gels and transferred onto blotting membranes. After incubation with antibodies against H3 and H3K9me3 and fluorescent detection, signal intensity was quantified using Image Studio Lite (LI-COR Biotechnology)). Additionally, time-lapse developmental kinetics of 130 transferred and cryopreserved embryos of 38 patient couples were analyzed.
Main results and the role of chance: No significant differences were found in patient characteristics between the pregnant and non-pregnant group. However, fertilization rate was found to be a confounding factor; no pregnancies occurred below a fertilization rate of 40% and only 4 occurred below a fertilization rate of 60%. The median H3k9me3/H3 ratio in the pregnant group was 1.01 (interquartile range (IQR): 0.69-1.16) and in the non-pregnant group 0.896 (IQR: 0.54-1.06), this difference was not significant. When using logistic regression analysis, adjusted for the fertilization rate (dichotomized as ≤60% or >60%), we observed a positive association between the H3k9me3/H3 ratio and ongoing pregnancy (odds ratio (OR) 6.43 (95% confidence interval (CI): 1.12-36.8; p-value 0.037). More importantly, none of the samples with a ratio of 0.5 or lower resulted in an ongoing pregnancy (n=7). After classifying sperm samples into quartiles according to the H3K9me3/H3 ratio, we observed that resulting embryos reached the 4-cell stage faster with sperm from to the lowest quartile of H3K9me3/H3 ratio, compared to the highest quartile (beta -4.4 hours, p-value 0.05). Our results point to an association between the H3k9me3/H3 ratio, embryo development and pregnancy outcome.
Limitations, reasons for caution: This initial analysis is performed with a relatively low number of samples. The OR of 6.43 has a wide 95% CI, probably due to low statistical power. Our observations await confirmation in a larger data set.
Wider implications of the findings: Semen analysis remains the cornerstone of male infertility diagnosis, despite its low prognostic value. Sperm epigenetic cHC inheritance likely has an important impact on early embryo development. Therefore, the H3k9me3/H3 ratio could serve as a parameter for sperm quality and aid in the prediction of pregnancy outcome.
Trial registration number: not applicable

P-581 The day of blastocyst biopsy and the chromosomal constitution. Evaluation of 5125 embryos by Next Generation Sequency (NGS)

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Study question: Is there a relationship between the day of blastocyst biopsy and the results NGS analysis?
Summary answer: Embryos biopsied on day 6 or 7 are associated with the increased probability of being an aneuploidy embryo and less likely to be mosaic embryo.
What is known already: There is controversy about whether an embryo that reaches the blastocyst stage on day 5 has a higher chance of being euploid than embryos which are biopsied later. In our study, chromosome constitution was evaluated by next-generation sequencing (NGS)-based preimplantation genetic testing for aneuploidy (PGT-A) and confounding factors were eliminated.
Study design, size, duration: Data was collected retrospectively from June 2016 to January 2020
Participants/materials, setting, methods: In total, 5125 blastocyst (day 5=2914, day 6 N=2154 and day7 N=57), generated from 1318 cycles were analysed with PGT-A. The chromosome constitution for each embryo was classified as euploid, aneuploid and mosaic. A multilevel model was made and associations betwwen variables by logistic regression were adjusted according to maternal age, SART blastocyst grade, fertilization method, biopsy operator and blastocyst stage.
Main results and the role of chance: The mean maternal age was 36.2 ± 4.2. Euploid rate was 62.1% and 37.9% (day 5 and day 6-7 respectively), aneuploidy rate was 47.0% and 53.0% (day 5 and day 6-7, respectively), mosaicism rate was 59.6% and 40.4% (day 5 and day 6-7, respectively) (p<0.001).

Embryos biopsied on day 6-7 have a significantly lower probability to be euploid and mosaicism than embryos biopsied on day 5 ((OR=0.76 [0.68-0.86]); (OR=0.84 (0.73 – 0.96) respectively) (p<0.001). On the contrary, embryos biopsy on day 5 were significantly more likely to be euploid than day 6-7 (OR=1.63[1.42-1.86]) (p<0.001).

Limitations, reasons for caution: The results observed in this study should be confirmed using a larger number of samples. For the NGS analysis, a chromosome with a variation between 20 to 80% was considered mosaic.

Wider implications of the findings: The present study revealed that embryos that reach blastocyst classified as full to hatched on day 5 are more like to be euploid compared to slow growing embryos.

Trial registration number: non-clinical trials

P-582 High level of concordance between invasive and non-invasive preimplantation genetic testing for aneuploidies (niPGT-A) at day5 and day6-7

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Study question: To explore ploidy concordance between invasive and non-invasive PGTA (niPGT-A) at different embryo culture time.

Summary answer: High level (>84%) of concordance rate for ploidy and sex, sensitivity (>88%), and specificity (76%) were obtained for both day6/7 samples and day5 samples.

What is known already: The analysis of embryo cell free DNA (cfDNA) that are released into culture media during in vitro embryo development has the potential to evaluate embryo ploidy status. However, obtaining sufficient quality and quantity of cfDNA is essential to achieve interpretable results for niPGT-A. More culture time is expected to be directly proportional to the release of more cfDNA. But embryo culture time is limited due to in-vitro embryo survival potential. Therefore, it is important to estimate the duration of the culture that will provide the maximum cfDNA that can be obtained without adversely affecting the development of the embryo.

Study design, size, duration: A total of 105 spent culture media (SCM) from day5-day7 blastocyst stage embryos have been included in this cohort study. The cfDNA of SCM samples were amplified and analyzed for niPGT-A by NGS analysis. The SCM samples were divided into 2 subgroups according the embryo culture hours (Day5 and Day6/7 group). The DNA concentration, informativity and euploidy results have then been compared with their corresponding embryos after trophectoderm biopsy (TE) and PGT-A analysis by NGS

Participants/materials, setting, methods: Embryos cultured until Day3 washed and cultured again in 20µl fresh culture media until embryo biopsy on Day5, 6, or 7. After biopsy SCM samples were immediately collected in PCR tubes and conserved at -20°C until whole genome amplification by MALBAC® (Yicon Genomics). The TE and SCM samples were analyzed by next-generation sequencing (NGS) using Illumina MiSeq® System. NGS data analysis has been done by Bluefuse Multi Software 4.5 (Illumina) for SCM and TE samples

Main results and the role of chance: Only the SCM samples which have an embryo with a conclusive result were included in this cohort (n=105). Overall 97.1% (102/105) of SCM samples gave a successful DNA amplification with a concentration ranging 32.4-128.5ng/µl. Non-informative (NI) results including a chaotic profile (>5 chromosome aneuploidies) were observed in 17 samples, so 83.3%(85/102) of SCM samples were informative for NGS data analysis. Ploidy concordance rate with the corresponding TE biopsies (euploid vs euploid, aneuploid vs aneuploid) was 84.7% (72/85). Sensitivity and specificity were 92,8% and 76,7%, respectively with no significant difference for all parameters for day 6/7 samples compared with day 5 samples. The false-negative rate was 3.5% (3/85), and false-positive rate was 11.7% (10/85).

Limitations, reasons for caution: The sample size is relatively small. Larger prospective studies are needed. As this is a single-center study, the impact of the variations in embryo culture conditions can be underestimated. Maternal DNA contamination risk cannot be revealed in SCM, therefore the use of molecular markers would increase the reliability.

Wider implications of the findings: Non-invasive analysis of embryo cfDNA analyzed in spent culture media demonstrates high concordance with TE biopsy results in both early and late culture time. A non-invasive approach for aneuploidy screening offers important advantages such as avoiding invasive embryo biopsy and decreased cost, potentially increasing accessibility for a wider patient population.

Trial registration number: Not applicable

POSTER VIEWING
REPRODUCTIVE ENDOCRINOLOGY

P-583 Differential lipidomic characteristics of children born to women with polycystic ovary syndrome

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Study question: To describe lipidomic characteristics of offspring born to polycystic ovary syndrome (PCOS-off) women and assess the associations of clinical phenotypes changes with differential lipids.

Summary answer: PCOS-off showed specific changes in lipidomics and some differential lipids (e.g., phosphatidylcholines, lysophosphatidylcholine and sphingomyelin) may be the potential markers of aberrant cardiometabolic health.

What is known already: Polycystic ovary syndrome (PCOS), the most prevalent endocrine disorder characterized by ovulatory dysfunction, hyperandrogenism and polycystic ovarian morphology, affects about 8–13% of women of fertile age. Aberrant metabolic pathophysiological changes and increased pregnancy complications associated with PCOS predispose PCOS patients to have suboptimal intrauterine environments and that may produce a detrimental impact on the cardiometabolic health of their children.

Study design, size, duration: A total of 141 blood plasma samples from 70 children born to PCOS women (43 girls, 27 boys) and 71 healthy control children (44 girls, 27 boys) were obtained for lipidomics.

Participants/materials, setting, methods: Blood samples were centrifuged at 2000 rpm, 4°C for 20 min, and the upper plasma was collected and used for lipid extraction. Then the waters ACQUITY UPLC I-Class system and The Xevo G2-S Q-TOF with an electrospray ionization (ESI) source (Waters, Manchester, UK) was used for chromatographic analysis and mass spectrometry analysis separately.

Main results and the role of chance: In total, 44 metabolites were found to be significantly altered in PCOS-off, including 8 up-regulated and 36 down-regulated metabolites. After stratified by sex, 44 metabolites were found to express differently in girls born to PCOS women (PCOS-g). 13 metabolites were up-regulated, and 31 metabolites were down-regulated, most of which belong to glycerolipids species. While 46 metabolites were found to express differently in boys born to PCOS women (PCOS-b) with 9 increased metabolites and 35 decreased ones, most of which were glycerophospholipids metabolites. Additionally, significant associations between metabolites changes and weight Z-score as well as high density lipoprotein level were found in PCOS-off. In PCOS-g, triglyceride, low density lipoprotein and high density lipoprotein level were found to be correlated with some metabolites, whereas in PCOS-b, thyroid stimulating hormone and high density lipoprotein were correlated with some lipids.

Limitations, reasons for caution: Other species of metabolites except lipids are not included in this study. Besides, some potential confounding maternal factors, such as smoking, drinking, breastfeeding etc. were not included due to the lack of data.

Wider implications of the findings: The results had broadened our understanding of PCOS-off's cardiometabolic status and emphasized monitor and special management in this susceptible group of population.

Trial registration number: not applicable

P-584 Female parental consanguinity is associated with a reduced ovarian reserve: a large observational study including 2198 women from the Arabian Peninsula

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Study question: Is parental consanguinity associated with reduced ovarian reserve in women from the Arabian Peninsula? Summary answer: Women descending from consanguineous unions have a reduced ovarian reserve compared with daughters of non-consanguine couples.
What is known already: Consanguineous marriage is defined as marriage between second-degree cousins or closer, with high prevalence in the Arabian Peninsula societies. An increased incidence of autosomal recessive diseases has been described in consanguineous marriages compared with non-consanguineous marriages. Despite the known adverse genetic impact of consanguinity, most available studies focus on the fertility of the consanguine couple. Only few publications, including low number of women, evaluated the impact of consanguinity on the fertility of their offspring, suggesting that daughters of consanguine parents might have reduced fertility associated to reduced ovarian reserve.
Study design, size, duration: A retrospective large-scale observational study was performed including 2482 women from the Arabian Peninsula who had their serum AMH and AFC measured as part of their fertility assessment at ART Fertility Clinics (UAE and Oman), from May 2015 to November 2019.
Participants/materials, setting, methods: 2482 women from the Arabian Peninsula, aged 19-50 years, were assessed. Consanguinity was defined as women whose parents were first-degree or second-degree cousins. Ovarian reserve was evaluated by Antral Follicle Count (AFC) with transvaginal ultrasound and serum AMH, measured by Elecsys (Cobas, Roche®) for all participants. Women with adnexal surgery history or/and hormonal treatment within previous three months (n=284) were excluded. Ethical approval was obtained from the Research Ethics Committee (REFA040).
Main results and the role of chance: After excluding women with previous adnexal surgeries, 2198 women were included for analysis. A total of 605 participants (27.53%) were descendants from consanguineous unions and 1593 (72.47%) reported non-consanguineous kinship of their parents. AMH and AFC (mean±SD) for the consanguineous group were 2.62±2.88 ng/mL and 12.78±9.73 antral follicles, respectively; and AMH and AFC (mean±SD) for the non-consanguineous group were 2.65±2.91 ng/mL and 13.07±9.39 antral follicles, respectively. Women from the consanguinity group were significantly younger (mean±SD: 33.74±6.64 years old) compared with the non-consanguinity group (mean±SD: 34.78±6.64 years old, $p<0.0001$). Both groups were similar in BMI (mean±SD: 28.63±5.46 versus 28.41±5.60 kg/m2, p=ns), years of infertility (mean±SD: 3.80±3.68 vs 4.04±3.79, p=ns), type of infertility (primary/secondary), dress code (Hijab/Niqab) and smoking status. As expected, AMH and AFC exhibit an age-dependent decline. To evaluate the differences on ovarian reserve between both groups, a multivariate analysis was performed including age, consanguinity and AMH/AFC. Women from the consanguine group showed significantly lower levels of serum AMH (R2=0.264, p=0.036) and AFC (R2=0.286, p=0.003) compared with non-consanguineous women, and the highest differences were found for women below 35 years of age (AMH p=0.035; AFC p=0.001).
Limitations, reasons for caution: Despite the large number of women included, the retrospective study design is a limitation. Results have to be treated with caution before translating into other populations, as these data are obtained from women native to the Arabian Peninsula, with high sociocultural/religious/ethnical similarities, which might differ to other consanguine populations.
Wider implications of the findings: Female parental consanguinity is associated with reduced ovarian reserve in the studied population, that might contribute to infertility. Future studies should examine the genetic and epigenetic basis of the current findings. Comprehensive clinical evaluation should include extensive family history and subsequent counselling of the affected couples.
Trial registration number: not applicable

P-585 The influence of omega-3 fatty acids on female fertility in assisted reproductive technology

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Study question: Is there evidence that the intake of omega-3 fatty acids has a positive effect on probability of clinical pregnancy in women undergoing IVF/ICSI treatment?
Summary answer: No significant correlation was found between omega- 3 fatty acid intake (neither supplemental or as fish intake) and clinical pregnancy.
What is known already: Omega-3 fatty acids are important substrates in metabolism and the supplement of omega-3 fatty acids have been thought to have a positive effect on semen quality in infertile men. Studies have shown that the intake of omega-3 fatty acids might also have a direct effect on oocytes, the quality of the embryos, and implantation of the embryo in the uterus at conception. However, the role of omega-3 fatty acids in female fertility still remains unclear as the relationship between omega-3 fatty acids and successful IVF treatment in women has shown conflicting results.
Study design, size, duration: Systematic review. Systematic literature research (PRISMA) on PubMed, Embase, and Cochrane identifying clinical studies focusing on omega-3 fatty acid intake amongst women receiving IVF treatment, using good scientific practice for literature search and management. 5 articles meeting inclusion criteria were found, with a total of 1.100 women undergoing IVF/ICSI treatment. Participants/materials, setting, methods: Population: women in fertility treatment Intervention: omega-3 fatty acid and/or fish intake Comparison: No/low intake of omega-3 fatty acid and/or fish intake Outcome: Primary: clinical pregnancy rate, Secondary: number of follicles, embryo quality, live birth rate
Main results and the role of chance: Number of follicles was found to be inversely associated to omega-3 intake in a single study. Higher levels of total omega-3 intake were found to be positively associated to embryo morphology scores, and thus quality, independent on energy intake. None of the four studies considering clinical pregnancy found any statistical significance in the association between fish intake and clinical pregnancy. Regarding live birth as an endpoint, fish intake, but not fish oil supplements, was seen to significantly increase the probability in one study with a dose-response relationship. A similar association was not found by the only other study also examining live birth. Limitations, reasons for caution: Only five studies were found to meet inclusion criteria. None of the included studies were randomized controlled trials. No meta-analysis was carried out due to the heterogeneity of the studies.
Wider implications of the findings: The need for further studies, including randomized controlled trials of high quality with larger population sizes, is critical in order to thoroughly investigate and conclude any possible association of the beneficial effect of omega-3 fatty acids on female fertility in regard to IVF treatment.
Trial registration number: Not applicable

P-586 Clinical outcome in frozen cycles using cryopreserved blastocysts derived from ovarian stimulation with follitropin delta

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Study question: To compare the live birth rate using frozen-thawed blastocysts obtained from ovarian stimulation with individualised follitropin delta dosing to conventional follitropin alfa dosing.
Summary answer: The live birth rate in cryo cycles conducted within 1 year after ovarian stimulation was comparable for individualised follitropin delta and conventional follitropin alfa treatment.
What is known already: It has been demonstrated that the follitropin delta (Rekovelle, Ferring Pharmaceuticals) in an individualised dosing regimen based

on anti-Müllerian hormone (AMH) level and body weight is non-inferior to conventional follitropin alfa (Gonal-f, Merck Serono) dosing with respect to ongoing pregnancy and ongoing implantation rates in fresh cycles. The individualised approach also reduced the risk of ovarian hyperstimulation syndrome (OHSS) versus the conventional approach. Furthermore, treatment with follitropin delta and follitropin alfa gave comparable pregnancy rates in repeated fresh cycles.

Study design, size, duration: Analysis of frozen cycles using blastocysts obtained from a randomised trial comparing follitropin delta versus follitropin alfa in 1,326 IVF/ICSI patients (18-40 years) and a subsequent trial of up to two additional ovarian stimulation cycles. The clinical outcome includes women with cryopreserved blastocysts following ovarian stimulation and who underwent frozen cycles within 1 year after starting stimulation in their last cycle.

Participants/materials, setting, methods: A total of 917 women had at least one Day 5 blastocyst which was vitrified and stored following up to three ovarian stimulation cycles. A started cryo cycle was defined as warming of a blastocyst. After warming, 1-2 blastocysts were transferred in cryo cycles, using natural cycle or programmed regimens. Treatment differences and 95% confidence intervals (CI) were calculated with adjustment for age strata and accounting for repeated cycles within patient.

Main results and the role of chance: The proportion of women with frozen blastocysts was similar in the two treatment groups, with 69.5% in the follitropin delta group and 68.8% in the follitropin alfa group. Similar postwarming blastocyst survival rates were observed for the two groups, with 87.4% of the warmed blastocysts proceeding to transfer in the follitropin delta group and 88.8% in the follitropin alfa group. About half of the women (48.1% in each treatment group) with frozen blastocysts underwent at least one frozen cycle with transfer within the 1-year period, with an average of 1.5 cycles per woman in the follitropin delta group and 1.6 cycles per woman in the follitropin alfa group. The ongoing implantation rate was 27.6% in the follitropin delta group and 27.8% in the follitropin alfa group (adjusted difference 0.5% [95% CI: -7.1%; 8.2%]). The live birth rate per started cryo cycle was 32.0% in the follitropin delta group and 31.3% in the follitropin alfa group (adjusted difference 1.2% [95% CI: -6.8%; 9.3%]), while the live birth rate per cryo cycle with transfer was 33.2% and 31.9% (adjusted difference 1.9% [95% CI: -6.2%; 10.0%]), respectively.

Limitations, reasons for caution: The number of blastocysts to be transferred in the frozen cycles as well as the protocol for endometrial preparation was based on local centre practices.

Wider implications of the findings: These findings suggest that the follitropin delta and follitropin alfa dosing regimens are equally effective in terms of live birth rate in frozen replacement cycles and add reassuring information to the clinical performance of cryopreserved blastocysts derived from ovarian stimulation with follitropin delta.

Trial registration number: NCT01956110, NCT01956123.

P-587 Highly purified human menopausal gonadotrophin (HP-hMG, Menopur) as a ready-to-use solution for injection in pre-filled pen is bioequivalent to HP-hMG powder for reconstitution

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Study question: Are serum FSH levels after single subcutaneous dosing of HP-hMG in a liquid formulation and a powder formulation bioequivalent?

Summary answer: The 90% CIs for the geometric mean ratios of serum FSH AUCt and Cmax were both within 0.8000-1.2500, thus the two formulations are bioequivalent.

What is known already: For several decades, HP-hMG (Menopur) has been used for the treatment of infertility; its efficacy and safety compared to other gonadotropins have been consistently demonstrated in several prospective, randomised controlled trials and meta-analyses (Deeks et al 2018; Bordewijk et al 2019). Menopur powder for reconstitution is available in multidose and single dose formulations. Up to 3 single dose vials (each containing 75 IU) may be dissolved into 1 mL solvent for administration. Recently, and for the first time, Menopur has been successfully formulated in a stable, ready-to-use solution for injection, which may be administered by a pre-filled pen.

Study design, size, duration: This was a randomised, two-way crossover, single dose, bioequivalence trial comparing Menopur liquid injected by pre-filled pen, with Menopur powder injected by conventional syringe and needle. The primary endpoints were AUCt and Cmax of baseline-adjusted FSH. Pituitary-suppressed, healthy women were randomised to receive one treatment sequence including a single subcutaneous injection of 450 IU Menopur liquid (600 IU/0.96 mL) and of 450 IU Menopur powder by two subcutaneous injections of 225 IU in 1 mL.

Participants/materials, setting, methods: Blood samples were collected pre- and post-dose, until 9 days after each injection. The PK parameters of FSH and hCG were assessed by noncompartmental methods with adjustment for endogenous pre-dose levels. Highly sensitive and specific electrochemiluminescence immunoassays were used for quantification and the LLOQ of the FSH and hCG assays were 1.47 mIU/mL and 0.5 mIU/mL, respectively, and the total validated CV was within 5% for both assays.

Main results and the role of chance: In total, 76 women were randomised and 56 completed the trial. The main reason for discontinuation was insufficient pituitary suppression prior to the second administration of HP-hMG. The mean FSH and hCG serum concentration-time profiles were comparable between the two HP-hMG formulations. The geometric mean ratios and 90% confidence intervals of FSH for HP-hMG liquid versus HP-hMG powder were 1.12 [1.0562; 1.1889] for AUCt and 1.17 [1.0946; 1.2490] for Cmax, showing that the two formulations were bioequivalent. Maximal serum FSH concentrations were reached at 18.19 h for HP-HMG liquid and 15.55 h for HP-hMG powder. In addition to FSH, the PK parameters for hCG were compared between the two HP-hMG formulations. The geometric mean ratios and 90% confidence intervals for HP-hMG liquid versus HP-hMG powder were 0.93 [0.86; 1.01] for AUCt and 0.94 [0.86; 1.02] for Cmax. There was no difference between the two groups in the incidence or severity of adverse events, and both preparations were well tolerated. Mild injection site reactions were less common after administration of HP-hMG liquid by a single injection compared to HP-hMG powder by two injections and were mostly related to pain and erythema after drug administration.

Limitations, reasons for caution: This bioequivalence study is based on the comparison of single dose administrations in healthy female volunteers of reproductive age.

Wider implications of the findings: The new HP-hMG solution for injection in a pre-filled pen will deliver the efficacy and safety of Menopur in a convenient delivery device.

Trial registration number: NA

P-588 Follicle-stimulating hormone receptor genotype and its influence on the results of double ovarian stimulation in IVF cycles

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Study question: Does the follicle-stimulating hormone receptor (FSHR) genotype influence the results of the ovarian stimulation treatment in the luteal phase?

Summary answer: All patients undergoing in-vitro fertilization benefit from luteal phase ovarian stimulation, regardless of their follicle-stimulating hormone receptor genotype.

What is known already: Previous studies suggest that FSH receptor polymorphism in position 680 influences the response to ovarian stimulation in the luteal phase. It was observed that patients with SS genotype seems to require a higher dose to obtain an optimal ovarian response. Later, it was reported that, in patients with SS genotype, a better performance seems to be obtained by administering highly purified urinary FSH while, in SN patients, better results were obtained with recombinant FSH. In patients with NN genotype, no differences were found. Our aim was to test whether this concept is applicable to ovarian stimulation in the luteal phase.

Study design, size, duration: One hundred and thirty-four patients were included in a retrospective study between July 2017 and September 2020. In these patients, a double stimulation protocol was carried out and the FSH receptor was genotyped either as part of the pre-treatment fertility tests or for the current study. Patients with a double stimulation treatment who could not be genotyped were excluded from the analysis.
Participants/materials, setting, methods: To genotype the 680 position of the FSH receptor, a real-time PCR for allelic discrimination was carried out using StepOnePlus™ Real-Time PCR System (Applied Biosystems™. Ref: 4376600). Non-parametic tests were used to study the differences between the groups. They were performed with the software R Statistical Software, version 4.0.3.
Main results and the role of chance: The results of ovarian stimulation in the luteal phase were better compared to the conventional follicular phase. Statistically significant differences ($p < 0.001$) were found in the number of retrieved oocytes (5.06 versus 3.51), retrieved MII (4.13 versus 2.91), fertilized oocytes (3.22 versus 1.81) and blastocysts formed (1.79 versus 0.62). Furthermore, these differences remained regardless of the genotype for the 680 position of the FSH receptor in all groups ($p < 0.05$).

In addition, better results were obtained in the luteal phase in patients who have been stimulated with the type of gonadotropin that already had better performance in the follicular phase for its genotype, that is, highly purified urinary FSH in SS patients and recombinant FSH in SN patients, compared to other types of gonadotropin ($p < 0.05$).

We also observed that stimulation in the luteal phase lasts longer and consume more gonadotropins than in the follicular phase. This is especially notable in the case of patients with SS genotype, who required slightly higher consumption of gonadotropins compared to the other genotypes in the luteal phase, as had previously been observed in the follicular phase for this genotype.
Limitations, reasons for caution: The retrospective study design and the sample size could be a limitation. Furthermore, we cannot determine whether the improvement in luteal phase performance is related to differences in the physiological environment between phases of the cycle or is caused by a possible activation of the ovary from the previous stimulation.
Wider implications of the findings: All patients undergoing in-vitro fertilization seems to benefit from luteal phase ovarian stimulation, regardless of their genotype for FSHR. In addition, the pharmacogenetic recommendation when choosing the type of FSH for ovarian stimulation should be the same both in the follicular phase and in the luteal phase.
Trial registration number: Not applicable

P-589 The effects of vanillic acid on production of testosterone and function of insulin in adipose and ovarian tissues in rat model of polycyctic ovary syndrome

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Study question: What is the possible therapeutic effects of vanillic acid (VA) on the treatment of Polycystic Ovary Syndrome (PCOS)?
Summary answer: Vanillic acid successfully alleviated metabolic and endocrine abnormalities in adipose and ovarian tissues in a rat model of PCOS.
What is known already: Polycystic Ovary Syndrome (PCOS), as a common endocrine disorder, is accompanied by hyperandrogenism, insulin resistance, ovulation problems, and infertility. In this study, the effects of vanillic acid (VA) on metabolic and endocrine abnormalities were evaluated in the adipose and ovarian tissues of a letrozole-induced rat model of PCOS.
Study design, size, duration: In this study, a letrozole-induced rat model was established and then the experimental groups were treated by VA and MET through oral gavage for one month. Before and after the treatment with drugs, the vaginal smear was carried out . Ovarian and adipose tissues were collected and frozen at −80 for further analysis following euthanasia of the rats.
Participants/materials, setting, methods: Thirty Six -week- old Wistar rats were divided into four groups (six rats per each group). Twenty-four rats were received letrozole for five weeks. Then, two experimental groups were treated by metformin (MET), and VA . Serum lipid profile, fasting glucose levels, and hormonal assays were done by corresponding commercial kits. The phosphorylation of AMP-activated protein kinase (AMPK) was detected by western blotting and the expression of studied genes were measured by Real-time PCR.
Main results and the role of chance: Our results showed that both VA and MET successfully reversed the abnormal estrous cycles of PCOS rats and reduced the serum testosterone levels and Steroid 17-alpha-hydroxylase/17,20 lyase (CYP17A1) gene expression. Furthermore, they improved Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and decreased the serum glucose and triglyceride levels, and gene expression of resisitin. The phosphorylation of AMPK was significantly decreased in adipose and ovarian tissues in PCOS group and both therapeutic factors successfully activated the AMPK in these tissues. However, VA had not such a significant effect in adipose tissue.
Limitations, reasons for caution: The main limitation of the current study was its design as a rat model, which may have limitation in the translation of knowledge to the clinics.
Wider implications of the findings: In summary, to the best of our knowledge, this study is the first study reported beneficial effects of VA on the treatment of PCOS. The findings of the current study shed light on an urgent need for discovering novel therapeutic pharmaceuticals regarding the treatment of the PCOS.
Trial registration number: 0

P-590 Women with hypothalamic hypogonadism have lower live birth rates following frozen embryo transfer

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Study question: How do live birth rates differ in anovulatory women with polycystic ovary syndrome and hypothalamic hypogonadism compared to normo-ovulatory women undergoing fresh or frozen embryo transfer?
Summary answer: Live birth rates are similar among all groups undergoing fresh embryo transfer but are significantly lower in women with hypothalamic hypogonadism undergoing frozen embryo transfer.
What is known already: Conflicting data exist regarding pregnancy outcomes in patients with tubal factor infertility versus polycystic ovary syndrome (PCOS). Some studies demonstrate higher pregnancy and live birth rates for women with PCOS undergoing fresh embryo transfer, but other studies demonstrate no difference. Women with PCOS have higher live birth rates than those with tubal factor infertility when undergoing frozen embryo transfer. Fewer data are available regarding IVF outcomes in women with hypothalamic hypogonadism (HH) and tubal factor infertility. Several studies report comparable live birth rates with fresh embryo transfer, but there are no data on frozen embryo transfer outcomes.
Study design, size, duration: Retrospective cohort study of all fresh and frozen autologous embryo transfers performed for patients with oligo-anovulation (PCOS, n=380 and HH, n=39) and normo-ovulation (tubal factor infertility, n=315) from 1/1/2012 to 6/30/2019. A total of 734 transfers from 653 patients were analyzed.
Participants/materials, setting, methods: Transfer outcomes, including implantation, miscarriage, clinical pregnancy and live birth rates, were assessed in fresh and frozen embryo transfer cycles. Adjusted relative risks (RR) and 95% confidence intervals (CI) were calculated adjusting for age, BMI, stimulation protocol, number of embryos transferred, embryo quality, endometrial stripe thickness and day of transfer. Poisson regression was used for counts and with an offset for ratios. Generalized estimating equations were used to account for patients contributing multiple cycles.
Main results and the role of chance: For fresh embryo transfer cycles, live birth rates are similar among patients with tubal factor infertility, PCOS and HH (29.5% vs. 37.9% vs. 35.9%, respectively, aRR 1.15 95% CI: 0.91-1.44 and aRR 1.23 95% CI: 0.81-2.00, respectively). When evaluating frozen embryo transfer cycles, patients with HH have lower live birth rates than patients with tubal factor infertility (26.5% vs. 42.6%, aRR 0.54 95% CI: 0.33-0.88) and patients with PCOS (26.5% vs. 46.7%, aRR 0.55 95% CI: 0.34-0.88). Additionally, patients with HH have higher chemical pregnancy rates and miscarriage rates than patients with tubal factor infertility (26.5% vs. 13.0% and 17.7% vs. 6.5%, respectively, RR 2.71

95% CI: 1.27-5.77 and RR 2.03 95% CI: 1.05-3.80, respectively). Point biserial correlation showed no significant correlation between live birth and endometrial stripe thickness in HH patients undergoing frozen embryo transfer (r = 0.028, p-value 0.876).

Limitations, reasons for caution: This study is limited by its retrospective nature and the small sample size of women with hypothalamic hypogonadism. Additionally, these data represent outcomes from a single academic center, so generalizability of our findings may be limited.

Wider implications of the findings: Lower live birth rates for HH patients undergoing frozen embryo transfer cycles are not correlated with endometrial stripe thickness. This may be due to absent gonadotropin signaling on endometrial receptors. A prospective randomized trial of HH patients to modified natural versus programmed frozen embryo transfer would best support this hypothesis.

Trial registration number: Not applicable

P-591 Mass spectrometry-based glycomic profiling of the follicular fluid total immunoglobulin G and proteome N-glycomes reveals deregulated inflammatory processes associated with specific controlled ovarian stimulation protocols

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Study question: Is there a significant inflammatory-related difference between analyzed follicular fluid (FF) glycome profiles regarding the ovarian stimulation protocol used in patients?

Summary answer: Observed differences between analyzed glycome profiles from patients that underwent different controlled ovarian stimulation (COS) protocols point to deregulated inflammatory processes associated with specific COS.

What is known already: Successful physiological folliculogenesis and ovulation require an adequate inflammatory response. On the other hand, COS application in ART relies on induced hormonal activation of systemic inflammatory processes. Several studies have confirmed a rise in inflammatory cytokines, CRP, and other markers of inflammation in patients subjected to different COS protocols, pointing to an enhanced inflammatory response during ovulation stimulation. Glycoproteins and glycans have an indisputable role in immune response modulation: proper glycosylation of glycoproteins plays a pivotal role in the regulation of normal physiological processes, and aberrant glycosylation of glycoproteins has been associated with various pathological states, including inflammation.

Study design, size, duration: Study design: Cross sectional – FFs from patients that underwent ART in modified natural cycle (MNC group) versus FFs from patients that underwent ART under GnRH antagonist COS (COS group). Size: 20 FFs from 20 patients undergoing ART. Duration: One year. Sampling procedure: Each FF was aspirated from the dominant follicle. In the COS group, only the fluid from the first aspirated follicle of each patient was collected.

Participants/materials, setting, methods: Study included 20 FF samples from 20 patients divided into two groups according to the applied ovarian stimulation protocol: MNC group (n=10) and COS group (n=10). The immunoglobulin G (IgG) was isolated from FF samples by immunoaffinity chromatography. The N-linked glycans derived from IgG molecule and the remaining FF total proteomes were enzymatically cleaved and subjected to derivatization procedure. N-glycomes of FF-isolated IgG and total proteomes were analyzed separately by MALDI-TOF-MS.

Main results and the role of chance: FF IgG N-glycome profiling The MALDI-TOF-MS based comparative analysis of the individual glycan relative abundances, revealed several significantly deregulated glycoforms between analyzed groups whose levels were significantly elevated (p 0.05) in the COS *vs.* MNC group. Furthermore, additional low abundant N-glycan species were also found to be deregulated between the analyzed groups: two monogalactolysed and monosialylated N-glycan compositions were only identified in the COS group. The comparative analysis of FF IgG N-glycome features revealed statistically relevant differences in the levels of two derived traits: galactosylation and bigalactosylation levels of the FF IgG N-glycome, both significantly downregulated (p 0.05) in the MNC *vs.* COS profile. Comparative analysis of FF total proteome N-glycome The majority of identified glycan compositions were complex type N-glycans representing more than 98% of the total N-glycome profiles in both analyzed groups. The comparative analysis of individual glycan relative abundances revealed relevant differences in regulation of ten N-glycan species between the two analyzed profiles. In the MNC group, six N-glycan species showed significantly increased abundances (p 0.05) compared with the COS group. Moreover, two compositions were exclusively identified in the MNC group, while two compositions were identified only in the COS group.

Limitations, reasons for caution: Since this preliminary study was conducted on relatively small sample size, all results should be verified on a larger sample set. Moreover, focused glycosylation analysis of a panel of individual FF acute phase blood serum derived proteins and immunoglobulins, might additionally clarify the inflammatory mechanisms underlying different ART stimulation protocols.

Wider implications of the findings: While glycome profiling of human FFs was conducted for the first time, previous evidence supports the shown association of aberrant inflammation in diverse ART stimulation protocols and in development of various pathological states (i.e. OHSS). Obtained results are in line with previous similar studies performed in the human plasma.

Trial registration number: uniri-biomed-18-161 1310

P-592 Safety and effectiveness of follitropin alfa biosimilar to originator follitropin alfa in real-world clinical practice: A Multinational Comparative, Prospective Cohort Study

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Study question: Are safety and effectiveness of Ovaleap® (follitropin alfa), and Gonal-f®, comparable in one treatment cycle of ART in routine clinical practice?

Summary answer: Safety in terms of incidence proportions of OHSS and OHSS severity, as well as pregnancy and live birth rates, were similar between Ovaleap® and Gonal-f®.

What is known already: Ovaleap® (Theramex), a r-hFSH, is a biosimilar medicinal product to Gonal-f® (Merck). As a biosimilar, it went through a rigorous series of physio-chemical, in vitro, in vivo tests and confirmatory Phase I and III studies, to demonstrate similarity/equivalence in quality, safety and efficacy to the reference medicinal product, per the European Medicines Agency (EMA) guidelines. Ovaleap® was approved by the EMA in 2013 for use at the same dose and for the same therapeutic indications as Gonal-f®. Further outcome data from a broader patient population on safety and live birth outcomes provides clinically important insights on newly introduced FSH medicines.

Study design, size, duration: SOFIA (**S**afety of **O**valeap® **F**ollitropin alfa in **I**nfertile women undergoing superovulation for **A**ssisted reproductive technologies) was a multi-national, comparative, non-interventional, prospective cohort study. The study was performed at 56 centers specializing in ART from six European countries, (Belgium, France, Germany, Italy, Spain, and the United Kingdom) from January 2017 to September 2019 and comprised of 817 infertile women undergoing controlled ovarian hyperstimulation in one treatment cycle for ART

Participants/materials, setting, methods: The study population comprised of infertile women undergoing controlled ovarian hyperstimulation for ART, who were administered Ovaleap® or Gonal-f® and were naïve to any FSH containing products. Eligible patients were enrolled at a ratio of approximately 1:1, both within and between countries. They were followed up to 30 days after the last FSH dose administration. Women who had a confirmed clinical pregnancy were followed until the end of the pregnancy or until delivery.

Main results and the role of chance: A total of 408 and 409 women who were administered Ovaleap® or Gonal-f®, respectively, were eligible for analysis. A total of 382 patients (94%) in the Ovaleap® and 390 patients (95%) in the Gonal-f® cohort completed FSH treatment (up to oocyte maturation triggering), respectively. The two cohorts were generally similar with regard to demographic and baseline characteristics. The incidence proportion of OHSS was 5.1% (95% CI: 3.4, 7.7) in the Ovaleap® and 3.2% (95% CI: 1.9, 5.4) in the Gonal-f® cohort. This difference in OHSS incidence proportion between the two cohorts was not statistically significant neither before (p=0.159) nor after univariate adjustment for each potential confounder (p>0.05). The incidence proportion of OHSS severity grades was similar in the two treatment groups (3.4% versus 2.0% for Grade I, 1.2% versus 1.0% Grade II, and 0.5% versus 0.2% Grade III, in the Ovaleap® and Gonal-f® cohorts, respectively) and without a significant statistical difference (p=0.865, for each grade). Among patients who had embryo transfer, clinical pregnancy rates were 33% and 31%, live birth rates 27% and 26% in the Ovaleap® and Gonal-f® cohorts, respectively.

Limitations, reasons for caution: Since treatment was non-randomised, the study may have been susceptible to selection bias. This was addressed at both the design stage, by balancing recruitment to a 1:1 ratio for Ovaleap® and Gonal-f® treatments, and also at the analysis stage in which, a univariate analysis was performed.

Wider implications of the findings: Findings from this first large European prospective comparative real-world SOFIA study demonstrated that effectiveness (pregnancy and delivery rates) and safety (risk and severity of OHSS), were similar between Ovaleap®and Gonal-f® treatments. Ovaleap, a biosimilar r-hFSH is therefore a suitable option for follicular stimulation in routine clinical practice.

Trial registration number: EUPAS17328

P-593 Self-monitoring of hormones via a urine-based hormonal assay — a topical endeavour into telemedicine in medically-assisted reproduction (MAR)

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Study question: How can cycle monitoring using a urine-based hormonal assay device improve current clinical practice in medically assisted reproduction (MAR)?

Summary answer: A urine-based hormonal assay has the potential to overcome the inconvenience of blood tests and reduce the frequency of appointments, waiting times and patient burden.

What is known already: Cycle monitoring via ultrasound and serum-based hormonal assays during MAR can provide information on the ovarian response and assist in optimising treatment strategies and reducing complications, such as ovarian hyperstimulation syndrome (OHSS). However, blood tests may cause inconvenience to patients due to repeated venepuncture and the need for frequent clinic appointments. Urine-based assays have been historically used by fertility specialists in clinics, but since got replaced by more practical and automated serum-based assays. Novel technology utilising rapid chromatographic immunoassay to test urinary reproductive hormones in a home setting could provide an alternative to current serum-based testing at clinics.

Study design, size, duration: A questionnaire was disseminated among 24 fertility specialists (2019-2020) on the use of ultrasound and serum-based hormone monitoring in clinical practice. In addition, the literature on the reliability of urine-based hormonal assays compared to serum-based hormonal assays during MAR was reviewed in order to examine if urine-based hormonal monitoring could be re-introduced in clinical practice using novel state-of-the-art technology.

Participants/materials, setting, methods: All 24 surveyed fertility specialists responded, representing 10 countries from across Europe, Asia and Latin America. Questions assessed the frequency and role of hormonal monitoring, the hormones tested and the drawbacks of blood tests. The PubMed search engine was used to search the Medline database for publications between 1960–2020 with (MeSH-) search terms related to cycle monitoring (e.g. fertility monitoring, controlled ovarian stimulation, ovulation confirmation) and hormonal assays (e.g. estrone-3-glucuronide or E1-3G).

Main results and the role of chance: The survey confirmed that many fertility practitioners (n=22/24) routinely conducted hormone monitoring during MAR, primarily for guiding dose adjustments (n=20/24) and indicating risk of OHSS (n=20/24). The reported drawbacks of blood tests included validity of results from different service providers, long waiting times and discomfort to patients due to travelling to clinics for tests and repeated venepunctures. The hormones routinely checked were E2 (n=22/22), P4 (n=18/22) and LH (n=15/22). The literature review revealed a relatively high correlation (correlation coefficients 0.85–0.95) between serum E2 and urinary E1-3G in gonadotrophin stimulated cycles (Lessing 1987, Catalan 1989, Rapi 1992 and Alper 1994). No studies assessed the correlation between serum P4 and urinary PdG or between serum LH and urinary LH in stimulated cycles. In natural cycles, the correlation coefficients between serum P4 and urinary PdG seemed to be slightly higher than those between serum E2 and urinary E1-3G (0.73–0.94 vs. 0.54–0.88) (Denari 1981, Munro 1991, Roos 2015, Stanczyk 1980). One study reported a moderate correlation coefficient (0.72) between serum and urinary LH in natural cycles (Roos 2015).

Limitations, reasons for caution: There is risk of selection-bias for fertility specialists included in survey, however, the 100% response rate is reassuring. The correlation coefficients between serum- and urine-based hormonal assay and the cost-effectiveness and time-efficiency of urinary assay should be confirmed in further clinical studies using a novel state-of-the-art remote urinary monitoring device.

Wider implications of the findings: Remote hormonal monitoring can be part of a novel digital health solution that includes remote ultrasound and tele-counselling to link clinics and patients at home. Especially during the unprecedented times of the COVID-19 pandemic, the prospect of remote monitoring system has the potential to improve patient experience during fertility treatment.

Trial registration number: Not applicable

P-594 Ovarian stimulation with luteinizing hormone supplementation: the impact of timing on ovarian response and ICSI outcomes

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Study question: Is there an impact of recombinant luteinizing-hormone (rLH) administration timing during controlled ovarian stimulation (COS) on ovarian response and intracytoplasmic sperm injection (ICSI) cycles outcomes?

Summary answer: rLH supplementation in patients with poor ovarian response (POR) improves laboratorial and clinical outcomes when started in the mid-follicular phase, in GnRH antagonist ICSI cycles.

What is known already: Meta-analyses demonstrated that the use of rLH combined with rFSH for COS may lead to more ongoing pregnancies than rFSH alone. However, there is limited evidence that the timing of rLH addition to rFSH may impact the ovarian response or the outcomes of ICSI, based on a limited casuistic, which demonstrated improved ovarian response, embryo quality and pregnancy rate with LH supplementation from GnRH antagonist administration day, in estimated POR patients. The objective of the present study was to further investigate this hypothesis in a larger population, and in subpopulations of patients stratified by age and response to COS.

Study design, size, duration: This historical cohort study included data obtained via chart review of 1278 ICSI cycles performed in 1278 patients between 2015 and 2018, in a private university-affiliated in vitro fertilization center. Post hoc power analysis was calculated, given α of 5%, sample size of

1278, and effect size for implantation rate. The achieved power was superior to 99%.

Participants/materials, setting, methods: Two groups were formed according to timing of LH administration: Group LH-start (n=323), in which LH was started on day-1; and Group LH-mid (n=955), in which LH was started with GnRH antagonist. Then, data were stratified according to female age (<35 years-old, n=283, and ≥35 years-old, n=995) and response to COS (poor response (POR): ≤4 retrieved oocytes, n=423, and normal response: >5 retrieved oocytes, n=855). Ovarian response and ICSI outcomes were compared among the groups.

Main results and the role of chance: In POR patients, significantly higher fertilization rate (68.3% ± 2.5 vs. 78.6% ± 3.7, p=0.023), blastocyst development rate (22.5% ± 7.2 vs. 44.7% ± 6.2, p=0.022) and implantation rate (17.6% ± 59.1 vs. 20.2% ± 43.2, p<0.001) were observed in Group LH-mid, even though the amount of LH used in these patients was not significant different from that used in Group LH-mid from patients with normal response to COS (1062.35 IU ± 54.33 vs. 925.81 IU ± 414.41, p: 0.431, respectively). For the general group and in patients aged ≥ 35 years, higher blastocyst development rates were observed in Group LH-mid compared to Group LH-start (33.0% ± 31.9 vs. 40.8% ± 32.6, p=0.012, and 28.8% ± 30.4 vs 38.5% ± 32.3, p=0.006, respectively). In patients aged < 35 years and in those with normal response to COS, similar outcomes were obtained irrespective of timing of LH administration.

Limitations, reasons for caution: The limitations included the retrospective design and limited sample size in subpopulations. In addition, the reduced clinical outcomes related to POR patients may hamper the true estimation of the differences between the stimulation groups in terms of pregnancy and miscarriage rates.

Wider implications of the findings: In POR patients, mid-follicular phase LH supplementation starting with 150 IU daily doses, may rescue the ongoing cycle by compensating an initial slow response, and balancing the deprivation of endogenous LH in GnRH antagonist cycles, with no need of expending more gonadotropin compared to patients with normal response to COS.

Trial registration number: Not applicable

P-595 Systematic dydrogesterone supplementation of artificial endometrial preparation cycles for frozen-thawed embryo transfer during Covid-19 pandemic: a good way to limit monitoring visits and optimize outcomes

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Study question: Does systematic dydrogesterone supplementation in artificial cycles (AC) for frozen-thawed embryo transfer (FET) during Covid-19 pandemic modify outcomes compared to prior individualized supplementation adjusted on serum progesterone (P) levels ?

Summary answer: Systematic dydrogesterone supplementation in AC for FET is associated with similar outcomes compared to prior individualized supplementation in patients with low P levels.

What is known already: In AC for FET using vaginal P for endometrial preparation, low serum P levels following P administration have been associated with decreased pregnancy and live birth rates. This deleterious effect can be overcome by addition of other routes of P administration. We obtained effective results by adding dydrogesterone to vaginal P and postponing FET by one day in patients with low P levels. However, in order to limit patient monitoring visits and to schedule better FET activity during Covid-19 pandemic, we implemented a systematic dydrogesterone supplementation without luteal P measurement in artificial FET cycles.

Study design, size, duration: This retrospective study aimed to analyse outcomes of 394 FET after 2 different protocols of artificial endometrial preparation. From September 2019 to Covid-19 lockdown on 15th March 2020, patients had serum P level measured on D1 of vaginal P administration. When P levels were < 11 ng/ml, dydrogesterone supplementation was administered and FET was postponed by one day. From May to December 2020, no P measurement was performed and dydrogesterone supplementation was systematically used.

Participants/materials, setting, methods: In our university hospital, endometrial preparation was performed using sequential administration of vaginal estradiol until endometrial thickness reached >7 mm, followed by transdermal estradiol combined with 800 mg/day vaginal micronized P started in the evening (D0). Oral dydrogesterone supplementation (30 mg/day) was started concomitantly to vaginal P in all patients during Covid-19 pandemic and only after D1 P measurement followed by one day FET postponement in patients with P levels <11 ng/ml before the lockdown.

Main results and the role of chance: During the Covid-19 pandemic, 198 FET were performed on D2, D3 or D5 of P administration with dydrogesterone supplementation depending on embryo stage at cryopreservation. Concerning the 196 FET before lockdown, 124 (63%) were performed after dydrogesterone addition from D1 onwards and postponement by one day in patients with serum P levels <11 ng/ml at D1 while 72 were performed in phase following introduction of vaginal P without dydrogesterone supplementation in patients with P >11 ng/ml. Characteristics of patients in the 2 time periods were similar for age (34.5 + 5 *vs* 34.1 + 4.8 years), endometrial thickness prior to P introduction (9.9 + 2.1 *vs* 9.9 + 2.2 mm), number of transferred embryos (1.3 + 0.5 *vs* 1.4 + 0.5) , embryo transfer stage (D2/D3/blastocyst: 8/16/76 % *vs* 3/18/79 %). No significant difference was observed between both time periods [nor between "dydrogesterone addition and postponement by 1 day" and "in phase" FET before lockdown] in terms of positive pregnancy test (39.4% *vs* 39.3% [44% *vs* 30.5%]), heartbeat activity at 8 weeks (29.3% *vs* 28% [29% *vs* 26.4%]) and ongoing pregnancy rates at 12 weeks (30.7% but truncated at end of October 2020 *vs* 25.5% [26.6% *vs* 23.6%]).

Limitations, reasons for caution: Full results of the Covid-19 period will be further provided concerning ongoing pregnancy rates as well as comparison of live birth rates and obstetrical and neonatal outcomes.

Wider implications of the findings: These results suggest that systematic dydrogesterone supplementation is as effective as individualized supplementation according to serum P levels following administration of vaginal P. This strategy enabled us to schedule easier FET and limit patient visits for monitoring while maintaining optimal results for FET in AC during the Covid-19 pandemic.

Trial registration number: not applicable

P-596 Association of oleic acid production in cumulus-granulosa cells with glutathione of in vitro matured oocytes

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Study question: Does oleic acid production in cumulus-granulosa cells affect glutathione levels of *in vitro* matured oocytes?

Summary answer: Oleic acid availability in cumulus-granulosa cells is associated with a higher glutathione level in *in vitro* matured oocytes.

What is known already: The monounsaturated fatty acid oleic acid is *de novo* synthesized by desaturation of stearic acid and can promote steroidogenesis and oocyte development *in vitro*. The endogenous antioxidant glutathione content in metaphase II oocyte is significantly higher than immature stages and is related to the normal oocyte maturation.

Study design, size, duration: Mouse germinal vesicles were co-cultured for 24 hours, during *in vitro* maturation, with granulosa cells treated with a specific inhibitor of oleic acid synthesis alone or in combination with oleic acid.

Participants/materials, setting, methods: Fluorescence staining was used to assess the glutathione content of mouse metaphase II oocytes following *in vitro* maturation as an indicator of cytoplasmic maturation. Glutathione was stained using Cell Tracker Blue –CMAC for 30 min at 37°C. After being washed in fresh media, stained oocytes were photographed by a fluorescence microscope. Cell area and associated fluorescence were quantified in 20 metaphase II mouse oocytes randomly chosen from *in vitro* matured oocytes for each condition.

Main results and the role of chance: The intracellular glutathione content was profoundly lower in metaphase II oocytes obtained from co-cultures with inhibitor-treated cumulus-granulosa cells than with the control cumulus cells (-50%, p<0.01). Oleic acid effectively recovered the negative effect of inhibitor on glutathione level nearly up to the level of the mock-treated cells.
Limitations, reasons for caution: The findings are limited to metaphase II. Measurement at more advanced stages of oocyte development is of interest. Inhibition of cellular fatty acid synthesis was performed solely with a specific chemical.
Wider implications of the findings: Involvement of the oleic acid availability for cumulus-granulosa cells in normal oocyte maturation may be of relevance in reproductive disorders, particularly in the pathological mechanism of impaired oogenesis.
Trial registration number: 400/3226

P-597 Placental expression of neurokinin B and its receptor NK3R is increased in women with polycystic ovary syndrome: results of a preliminary study

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Study question: Is the placental expression of neurokinin B (NKB) and its receptors NK1R, NK2R and NK3R affected by the polycystic ovary syndrome (PCOS)? Summary answer: The placental expression of NKB and NK3R is increased in PCOS, while the expression of NK1R and NK2R is not affected. What is known already: Women with PCOS are at increased risk of pregnancy complications and poor pregnancy outcomes. Defective placentation is among the proposed mechanisms involved. Altered NKB placental expression has been associated with several conditions characterized by placental dysfunction, such as pre-eclampsia and intra-uterine growth retardation. To our knowledge, the expression of NKB and its receptors has not been studied in placental tissue of women with PCOS.
Study design, size, duration: This was a single-center, prospective, case-control study. Women with PCOS according to the Rotterdam criteria (cases) and healthy pregnant women (controls) were enrolled at first prenatal visit and followed until delivery. Only women with spontaneous conception and singleton, uncomplicated, term pregnancies (10 PCOS and 10 controls) were included in the final analysis. All participants provided informed consent.
Participants/materials, setting, methods: At delivery, placental specimens were collected and immediately submerged in RNAlater solution. Samples were stored at -20oC until analysis. The mRNA expression of NKB, NK1R, NK2R and NK3R was quantified by real-time PCR (RT-PCR). The relative mRNA expression was estimated by the ΔΔCT method, using β-actin as reference (housekeeping gene). Statistical analysis was performed using SPSS 25.0, and the level of statistical significance was set at 0.05 (two-sided).
Main results and the role of chance: The placental mRNA expression of NKB and NK3R was significantly higher in PCOS women versus controls (2.4-fold, p<0.05 for NKB and 7-fold, p<0.05 for NK3R). No significant alterations were observed in the mRNA expression of NK1R and NK2R between the two groups. There was no statistically significant difference regarding age, BMI, caesarian section frequency, offspring sex and birth weight between women with PCOS and controls. The placental expression of NKB and its receptors was correlated neither with maternal age and BMI, nor with offspring birth weight.
Limitations, reasons for caution: The main limitation of this study is the small sample size. Expanding the number of participants is the necessary next step, in order to corroborate our preliminary findings. Furthermore, correlations between the placental expression of NKB, NK1R, NK2R, NK3R and maternal sex steroids, glucose and insulin levels should be sought.
Wider implications of the findings: The present study is the first to demonstrate increased placental expression of NKB and its receptor NK3R in women with PCOS. These findings support a potential role for NKB as a mediator of placental alterations characterizing PCOS.
Trial registration number: not applicable

P-598 Further evidence for a functional hormonal adrenal-ovarian axis affecting female infertility

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Study question: How does here presented case offer further evidence for existence of a functional hormonal adrenal-ovarian axis?
Summary answer: This is the first case of iatrogenic Cushing syndrome leading to severe adrenal and ovarian insufficiency, as evidenced by undetectable estrogen and low androgen levels.
What is known already: Animal models and human data have convincingly demonstrated that hypo-androgenism affects follicle recruitment and growth, especially at small growing follicle stages, in most severe cases even mimicking primary ovarian insufficiency (POI). In milder forms, hypoandrogenism reduces follicle number, egg numbers as well as egg quality, unless reconstituted in timely fashion before IVF cycle start.
Study design, size, duration: We here report a 34-year-old G1P1, who presented for a second opinion with a diagnosis of secondary "unexplained" infertility after two IVF cycles at another fertility center.
Participants/materials, setting, methods: Since our center considers a diagnosis of "unexplained" infertility as subjective, the patient underwent a thorough diagnostic evaluation. She was using oral contraceptive pills for one week at the time her laboratory results were drawn. Main results and the role of chance: Her free (FT) and total testosterone (TT) (0.4 pg/ml and 5.0 ng/dL, respectively), DHEA and DHEAS (103.0 ng/dL and 92.0 µg/dL, respectively) were low and her estradiol was undetectable (<25 pg/mL), reflecting significant adrenal as well as ovarian suppression. Morning ACTH was undetectable at <5 pg/mL but cortisol was abnormally elevated (17.7mcg/dL), leading to diagnoses of secondary adrenal insufficiency as well as secondary ovarian insufficiency (SOI) due to adrenal hypo-androgenism from lack of ACTH production. She, in addition, revealed a positive ANA titer (1:160). Because of eczema, she for over a year had been on a super-potent topical steroid ointment. Upon termination of this steroid, adrenal as well as ovarian function, as evidenced by her hormonal values, normalized.
Limitations, reasons for caution: This is the first case in the literature where iatrogenic-induced insufficiency of adrenal androgen production resulted in secondary ovarian insufficiency (SOI), characterized by undetectable estradiol, reversible by withdrawal of topical steroid treatment.
Wider implications of the findings: This case offers further evidence that the traditional hypothalamic-pituitary-ovarian axis (HPAA) extends downstream to ovaries (HPAOA), reaffirming the ability of adrenals to control ovarian function.
Trial registration number: n/a

P-599 random antral follicle count, performed at any day of the menstrual cycle, demonstrates the same predictive value for ovarian response in in vitro fertilization cycles

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Study question: Does antral follicle count (AFC) retains its predictive value for ovarian response to stimulation for in vitro fertilization (IVF) throughout the whole menstrual cycle?
Summary answer: AFC is strongly correlated to anti-mullerian hormone (AMH) and highly predictive of good ovarian response whatever the day of cycle the ultrasound is performed.

What is known already: Usually performed in the early follicular phase (at day 2-3 of the menstrual cycle), AFC and AMH are the most accurate markers of ovarian reserve. They are routinely used to predict ovarian response to ovarian stimulation for IVF and eventually to individualize the gonadotropin starting dose.
Study design, size, duration: Retrospective cohort study performed between January, 2017 and December, 2019.
Participants/materials, setting, methods: 410 consecutive women aged 20 to 42 years were included. Random AFC (r-AFC) was performed during the fertility workup whatever the day of their menstrual cycle was: early follicular phase i.e. day 1 to day 6 (eFP-AFC), mid follicular phase i.e. day 7 to 12 (mFP-AFC) and luteal phase i.e. day 13 or after (LP-AFC). A second AFC was performed before the start of the stimulation (SD1-AFC). AMH was measured in the early follicular phase.
Main results and the role of chance: Random AFC (r-AFC) was correlated to AMH (r=0.692; p<0.001), SD1-AFC (r=0.756; p<0.001) and number of oocytes retrieved (r=0.491; p<0.001). When regarding AFC depending on the cycle day group, the correlation with AMH was significantly higher for the LP-AFC, (LP-AFC) (r=0.853) than for the eFP-AFC (r=0.657; p<0.001) and for the mFP-AFC (r=0.668). The correlation with SD1-AFC was similar regardless of the time of performance of r-AFC (r=0.739, 0.783, 0.733, respectively for eFP, mFP and LP-AFC). Moreover, the ROC analysis showed the same predictive value for good ovarian response (more than 6 oocytes retrieved) for the eFP-AFC, mFP-AFC and LP-AFC (AUC 0.73, 0.75 and 0.84 respectively) as well as for AMH and SD1-AFC (AUC 0.74 and 0.74, respectively).
Limitations, reasons for caution: This is a retrospective analysis, however data were prospectively collected and the method for ultrasound acquisition of AFC was standardized.
Wider implications of the findings: The absence of significant variation of AFC across the menstrual cycle allows to its random performance. Ultrasound performed besides early follicular phase discloses informations on ovaries, the uterus and the endometrium. It is more comfortable and convenient for women and physicians by limiting targeted appointment during menstruation and reiterated examination.
Trial registration number: not applicable

P-600 Ovarian function following intraovarian injection of autologous platelet rich plasma (APRP) in women with low functional ovarian reserve

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Study question: Does exposure of ovaries to autologous growth factors in platelet rich plasma (APRP) affect the pituitary ovarian axis?
Summary answer: Within 60 days after injection, growing follicle numbers and estrogen levels increased, though FSH did not change, with effects most pronounced in still menstruating women.
What is known already: APRP is extracted from a patient's autologous blood and delivers growth factors. It is widely used in several medical specialties and has in infertility practice been reported to increase folicle/egg numbers if injected into ovaries and improve endometrial thickness/implantation if used for perfusion of the endometrium.
Study design, size, duration: Prospective observational cohort study of women with low functional ovarian reserve, followed for 60 days after subcortical injection of ovaries.
Participants/materials, setting, methods: 44 women with prior poor response to ovulation induction, FSH > 12 mIU/mL and AMH < 1.0 ng/mL. APRP was prepared using Regen Lab PRP Kit which is approved by the US-FDA. 1.0-1.5 ml of PRP was injected into the cortex of each ovary divided among 7 to 10 injection sites. Participants were followed every three days with monitoring for estradiol, FSH and follicle growth for the first two weeks after PRP and then weekly.
Main results and the role of chance: 21/43 patients still regularly menstruated (subgroup A, age 43.9 ± 5.1 years); 23/43 (subgroup B, age 42.6 ± 6.2 years) were amenorrheic for a median of 6 months. In A, AMH, FSH and estradiol were 0.18 ± 0.20 ng/mL, 37.5 ± 47.6 mIU/mL, and 100.2 ± 73.4 pg/mL, while in B they were 0.06 ± 0.11 ng/mL, 73.0 ± 44.8 mIU/mL and 66.7 ± 57.6 pg/mL. Following APRP, A-patients demonstrated increased estradiol to 211 ± 193.7 pg/mL (P= 0.029) while B-patients only demonstrated a trend to 98.1 ± 86.5 (P=0.09). Among A patients, 14/21 (66.7%) entered IVF cycles and 5/21 (23.8%) reached retrieval. So-far 1 patients established an ongoing clinical pregnancy. Among B patients 8/23 (34.8%) entered IVF cycles and only 2/23 (8.7%) reached retrieval and none achieved pregnancy.
Limitations, reasons for caution: This observational study was only carried out to estimate possible effects of APRP treatments. Based on these observations, we are now conducting a randomized controlled trial, limited to cycling women under age 45 years [registration # NCT04278313].
Wider implications of the findings: PRP appears to have limited ability to affect ovarian reserve of older, and especially amenorrheic women. It may, however, exert more favorable effects on still menstruating women. Promotion of APRP treatment as "ovarian rejuvenation," however, appears to be an inappropriate choice of words.
Trial registration number: N/A

P-601 Anovulatory patients with PCOS have lower euploidy rates compared to those with hypothalamic amenorrhea and to normo-ovulatory patients

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Study question: How do euploidy rates differ in anovulatory women with polycystic ovarian syndrome (PCOS) and hypothalamic hypogonadism (HH) compared to normo-ovulatory women undergoing IVF/ICSI?
Summary answer: Patients with PCOS have a significantly lower euploidy rate compared to patients with HH and patients with tubal factor infertility.
What is known already: Previous studies have demonstrated similar blastocyst conversion rates in women with PCOS and tubal factor infertility. Reported aneuploidy rates in preimplantation genetic testing cycles are similar in women with PCOS and tubal infertility. There are no data on blastocyst conversion or aneuploidy rates in women with HH. While PCOS and HH are different physiologic processes, patients with these disorders are reported together to SART and to the CDC National ART Surveillance System under the diagnosis of "ovulatory dysfunction". Study design, size, duration: Retrospective cohort study of all autologous IVF and ICSI cycles for patients with oligo-anovulation (PCOS, n=552 and HH, n=48) and normo-ovulation (tubal factor infertility, n=423) from 1/1/2012 to 6/30/2019. A total of 1023 cycles from 720 patients were analyzed.
Participants/materials, setting, methods: Cycle outcomes, including number of oocytes, mature oocytes, blastocysts and euploid blastocysts were assessed for each diagnosis. Adjusted relative risks (aRR) and 95% confidence intervals (CI) were calculated adjusting for age, BMI, AMH, and stimulation protocol. Poisson regression was used for counts and with an offset for ratios. Patients contributing multiple cycles were accounted for using general estimating equations.
Main results and the role of chance: PCOS patients were given a lower starting dose of gonadotropins and received less total gonadotropins compared to patients with tubal factor infertility or HH, but had similar stimulation durations as tubal-factor patients. Patients with HH received higher total doses of gonadotropins and had longer stimulation durations. PCOS patients had significantly more oocytes retrieved and a higher number of blastocysts than patients with tubal factor infertility (18.9 vs. 13.6 aRR 1.16 95% CI: 1.05-1.28 and 6.6 vs. 3.7 aRR 1.32 95% CI 1.10-1.57, respectively). Patients with HH had a similar number of oocytes retrieved and number of blastocysts compared to tubal factor patients. The blastocyst conversion rate was higher for PCOS than tubal (59.4% vs. 49.7%), but not significantly different (aRR 1.04 95% CI: 0.94-1.15). Blastocyst conversion and euploidy rates were similar for HH and tubal factor patients (51.9% vs. 49.7% and 39.1% vs. 44.9%, respectively, aRR 1.01 95% CI: 0.81-1.26 and aRR 1.05 95% CI: 0.85-1.31, respectively). In the adjusted model, patients

with PCOS had a significantly lower euploidy rate than patients with tubal infertility (aRR 0.75 95% CI: 0.58-0.96). Patients with HH also had a significantly higher euploidy rate compared to women with PCOS (aRR 1.41 95% CI: 1.05-1.89).
Limitations, reasons for caution: This study is limited by its retrospective nature and the small sample size of women with hypothalamic hypogonadism. Additionally, these data represent outcomes from a single academic center, so generalizability of our findings may be limited.
Wider implications of the findings: Cycle outcomes differ for ovulatory dysfunction patients with PCOS as compared to those with HH. HH patients require higher total doses of gonadotropins and longer stimulations to achieve similar cycle outcomes as normo-ovulatory patients. While PCOS patients have more embryos, the percent of euploid blastocysts is lower.
Trial registration number: Not Applicable

P-602 Pregnancy outcomes of progestin primed ovarian stimulation protocol, GnRH antagonist protocol and GnRH agonist protocol for young patients undergoing PGT-M

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Study question: To investigate the pregnancy outcomes of progestin primed ovarian stimulation protocol, GnRH antagonist protocol and GnRH agonist protocol for young patients undergoing preimplantation genetic testing for monogenic gene diseases.
Summary answer: PPOS protocol could reduce the normal chromosome formation and further development potential of embryos, suggesting that the PPOS protocol should be used cautiously.
What is known already: GnRH antagonist protocol (GnRHant) and GnRH agonist protocol (GnRHa) have been used in clinic for many years as routine regimens, and their ovarian stimulation effects and pregnancy outcomes have been confirmed by a large number of literatures. As a new protocol in recent years, the reports of pregnancy outcomes of progestin primed ovarian stimulation protocol (PPOS) are inconsistent.
Study design, size, duration: This retrospective cohort study was performed in a reproduction center from a tertiary hospital between September 2018 and November 2020 which included 147 young patients (<35 year old) undergoing preimplantation genetic testing for monogenic gene diseases (PGT-M) after stimulated by progestin primed ovarian stimulation protocol (n=44), GnRH antagonist protocol (n=60) or GnRH agonist protocol (n=43).
Participants/materials, setting, methods: This study included 147 young patients (<35 year old) undergoing preimplantation genetic testing for monogenic gene diseases (PGT-M) after stimulated by progestin primed ovarian stimulation protocol (PPOS, n=44), GnRH antagonist protocol (GnRHant, n=60) or GnRH agonist protocol (GnRHa, n=43). The primary outcomes were normal karyotype embryo rate and live birth rate. The embryological and clinical outcomes were measured.
Main results and the role of chance: Basic characteristics such as infertility duration, age, and body mass index (BMI) were comparable in study groups. No significant difference was found in the number oocytes retrieved or viable embryos between the groups.

Normal karyotype embryo rate of PPOS protocol was significantly lower than GnRHant and GnRHa protocol (57.6% for PPOS vs 76.0% for GnRHant vs 67.3% for GnRHa).

No significant difference were found in chemical pregnancy rate (77.3% for PPOS vs 73.3% for GnRHant vs 74.4% for GnRHa) or clinical pregnancy rate (69.8% for PPOS vs 71.7% for GnRHant vs 72.5% for GnRHa). While live birth rate of PPOS protocol was significantly lower than GnRHant and GnRHa protocol (45.5% for PPOS vs 58.3% for GnRHant vs 72.2% for GnRHa).
Limitations, reasons for caution: This is a preliminary study which needs to be further confirmed by large-scale clinical studies.
Wider implications of the findings: Although this is a preliminary study which needs to be further confirmed by large-scale clinical studies, the current results suggest that the application of PPOS should be cautious.
Trial registration number: -

P-603 Atretic eggs - frequency and factors which increase their production

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Study question: To investigate the factors which are associated with higher number and share of atretic oocites (AO) such as quintative ovarian reserve, gonadotropin doses, age, BMI, smoking, pelvic surgery. Summary answer: There are convincing data that factors as advanced age, overweight, smoking and pelvic surgery are related to higher share of AO, while stimulation doses not.
What is known already: Atretic eggs are cells that have different deviations in morphology - dark or granular cytoplasm, cytoplasmic fragments, dark area of the pellucid, large perivithelial space, abnormalities in shape and are usless. There is no consensus on the reasons for their formation. Studies demonstrate that combined estimation of the quantitative and qualitative reserve of the ovary is difficult, the transformation of primordial follicles into antral takes months in which the cohort of antral follicles and gametes changes. There are speculations of the likely negative impact of lifestyle factors t like smikong, obesity, age. Other blame higher doses of gonadotropins.
Study design, size, duration: This is a 3 year retrospective study on 2721 IVF/ICSI cycles of controlled ovarian hyperstimulation with long or antagonist protocols. The mean number and share of AO of all oocites retrieved were calculated in order to investigate there relation to factors like ovarian reserve, gonadotropin doses, age, BMI, smoking, history of pelvic surgery. Participants/materials, setting, methods: Depending on the factors investigated, the study groups were formed as follows: = ovarian reserve - <5antral folicles (AF) (n=307), 5-10AF(n=994), > 10AF(n=584) = stimulation doses -1500E (n=365),1500–3000E(n=790), 3000–4500(n=264), > 4500E(n=34) = age - ≤ 30(n=391), 31–34(n=467), 35–39(n=679),≥ 40(n=412) = BMI - <18.5(n=109), 18.5-24.9(n=668), 24.9-30(n=277), >30(n=111) = smoking - (n=431), nonsmoking (n=286) = pelvic surgery - (n=572), without surgery (n=630).
Main results and the role of chance: Regarding the ovarian reserve the mean number of AO rises significantly *(H=59.7,* ***p<0.0001****)* in paralel with the rise of all oocites retrieved, but the shre of AO stays same in each group *(H=0.39, p=0.828)*. As regard of the influence of doses of gonadotropins on the share of AO, there is no difference related to the increase of doses *(H=1.69; p=0.640)* - it is comparable,15-20%. The findings concerning age are interesting - the total number of eggs retrieved by age expectedly decreases but the share of AO is same between groups *(H=4.8, p=0.185)*, around 20%. At the same time in the group of women with only AO retrieved, t43,1% are above 40 years. Overweight and smoking are strongly related to the higher share of AO in obese and smoking women - *(H=11.4;* ***p=0.010****)* and *(U=54 342****; p=0.005****)* respectively. In addition among women with only AO, 73,9% are smoking *(c2 =5.26;* ***p=0.022****)*. Regarding the influence of pelvic surgery on quality of eggs, data shows higher share of AO among operated one is18% (U=*165815;* **p=0.012**), probably due to inflammatory processes in the pelvis.
Limitations, reasons for caution: It is possible same women to be prsent in different study groups.
Wider implications of the findings: Increse of stimulation gonadotropins increse the number of eggs retrieved and respectively the chances for pregnancy without compromising the quality of eggs. An increase in the share of AO are related to age, overweight, smoking,pelvic surgery in in the pelvis. These findings suggest preventive measures to preserve women's fertility potential.
Trial registration number: Not aplicable

P-604 Effectiveness comparison of antral follicular count (AFC), follicular-output-rate (FORT), follicle-to-oocyte-index (FOI), oocyte-sensitivity-index (OSI), and follicular-sensitivity-idex (FSI) for predicting clinical pregnancy rates in IVF

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Study question: Which is better for predicting clinical pregnancy rate : AFC, FORT, FOI, FSI, or OSI?
Summary answer: Both AFC and OSI can be used to predict clinical pregnancy better than FORT, FOI or FSI.
What is known already: AFC, FORT, FOI, OSI, FSI can be used to predict clinical pregnancy, but no study compared which one is better
Study design, size, duration: Retrospective study using data from medical record (2016-2018) Subjects were patients underwent IVF cycle at Aster Clinic in Hasan Sadikin Hospital Bandung. Subjects divided into 2 groups: clinically pregnant that is visible gestational sac on ultrasound (n = 83) and not pregnant (n = 148). Inclusion criteria : antagonist protocols, <45 years, basal follicle stimulating hormone (FSH) ≤ 12 IU/L, ICSI fertilization method, and fresh transfer cycle.
Participants/materials, setting, methods: AFC categorized < 5 and ≥ 5 (poseidon) FORT=pre-ovulatory follicles(16-20 mm) x 100 divided by AFC(2-10 mm). FOI=oocytes obtained x 100 divided by AFC. OSI=oocytes obtained x 1000 divided by total FSH dose. FSI=pre-ovulatory follicles x 100,000 divided by (AFC x total FSH dose). FORT and FSI divided using percentil 33 and 67. OSI divided into 3 groups by cut-off 1.697/IU for poor-response and 10.07/IU for hyperresponse. FOI divided into 2 groups, ≤ 50% or > 50%
Main results and the role of chance: Group of AFC ≥ 5 had a significantly higher clinical pregnancy rate than the AFC < 5 group (39.49 % vs. 16.67 % ; p = 0.009). High and moderate OSI had higher clinical pregnancy rate than low OSI (66.37 % vs. 37.72 % vs. 25.45 % ; p = 0.038). There is a significant negative correlation between OSI and age (-0.454) or total FSH dose (-0.594). There is a significant positive correlation between OSI and AFC (0.625), the number of follicles at trigger (0.792), and oocytes (0.923). There were no significant differences in clinical pregnancy rates between the FORT, FOI, and FSI groups.
Limitations, reasons for caution: Limitation : Retrospective study using medical record data Ultrasound measurement was done by many reproductive gynecology specialist (not 1 person) --- observer bias.
Wider implications of the findings: This study found no association between FORT, FOI, FSI on clinical pregnancy. Why?

- FORT, FSI, FOI use measurement number of follicles at trigger and antral follicle.
- Differences among observers in interpreting antral follicles and number of follicles at trigger, or inaccurate measurement.
- No FORT, FOI, and FSI cut off values from previous study.

Trial registration number: Not applicable

P-605 Low serum progesterone on the day of frozen embryo transfer after artificial endometrial preparation: exploring the clinical impact of "rescue" strategies

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Study question: Can we rescue treatments with low progesterone (PG) levels the day of frozen embryo transfer (FET) by adding subcutaneous progesterone?
Summary answer: After receiving additional supplementation with subcutaneous progesterone, women with low serum progesterone on cryotransfer day, have similar ongoing pregnancy rates as women with normal levels.
What is known already: Micronized vaginal progesterone fails to achieve optimal serum levels in up to 30% of patients receiving frozen embryos under artificial cycles (AC) despite the administration of 400 mg twice daily. Cancelling the thawing process and restarting a new treatment is a very disappointing option for patients and doctors. An alternative strategy is to administrate additional progesterone subcutaneously. The efficacy of the additional administration of subcutaneous progesterone as a "rescue" strategy in terms of clinical outcomes remains to be validated.
Study design, size, duration: We included 356 FET performed at Instituto Bernabeu between January 2019 - August 2020 in a retrospective case-control study. Groups were established according to PG levels on the day of the embryo transfer. The Control Group included: patients with optimal progesterone levels (≥8.8 ng ml); while the Rescue Group included those with suboptimal progesterone levels (<8.8 ng ml).
Participants/materials, setting, methods: All patients performed frozen embryo transfer after artificial endometrial preparation. All embryo transfers were performed at blastocyst stage after 5 days of progesterone administration. Progesterone levels were assessed the day of the embryo transfer by an electrochemiluminescence immunoassay. Samples were obtained 2-5 hours after the last vaginal progesterone administration.

Primary outcome was Ongoing Pregnancy Rates (OPR). Secondary outcomes were pregnancy rates (PR), miscarriage rates (MR) and biochemical miscarriage (BM).
Main results and the role of chance: 301 patients were included in the Control Group and 55 in the Rescue Group. No significant differences were found between both groups. OPR rate was 34.7% for patients in the control group versus 26.4% in the rescue group (p=0.240)

PR was 52.5% for patients with optimal PG levels vs 54.5% when PG levels were below 8.8 ng/mL. Both BM and MR tend to be higher in women who had low serum PG: BM (21.4% vs 15.5%) and MR (28.6% vs 18.1%), without reaching significant statistical difference.

In addition, we analyzed data from a sub-group of patients who received extra subcutaneous progesterone (based on clinician´s decision), despite having normal serum PG levels. No differences in clinical outcomes between these groups were observed either. OPR was 29%, vs 35.4% (p=0.241), PR was 51.8% vs 53.7%; BM was 16.7% vs 16.3% and MR was 26.9% vs 17.1% between women who received an extra subcutaneous PG dose versus women who did not, respectively.

Weight and BMI distribution were homogeneous across groups. A discreet difference was observed in age distribution (control group mean age 41.6 years vs. 39.7 years in the rescue group).
Limitations, reasons for caution: The retrospective collection of data and a limited sample size constitutes the main limitations of the study. Significant statistical differences were not found between groups but still differences might be clinically relevant. Larger studies are needed to reach robust conclusions on the strategy.
Wider implications of the findings: In AC cycles, when supplemented with additional subcutaneous progesterone, women showing low serum progesterone on cryotransfer day may expect similar clinical outcomes as women with normal levels. Pending on confirmatory studies, this strategy could consider as an alternative to cycle cancellation.
Trial registration number: Not applicable

P-606 A second stimulation in the same ovarian cycle rescues advanced-maternal-age patients obtaining ≤ 3 blastocysts after the conventional approach by preventing treatment-discontinuation

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Study question: Is double stimulation in the same ovarian cycle (DuoStim) a valuable strategy to rescue advanced-maternal-age patients obtaining ≤ 3 blastocysts for chromosomal-testing after conventional stimulation?
Summary answer: DuoStim is effective to prevent treatment discontinuation thereby increasing the 1-year cumulative-live-birth-rate among advanced-maternal-age patients obtaining 0-3 blastocysts after a first conventional stimulation.
What is known already: Folliculogenesis is characterized by continuous waves of follicular growth. DuoStim approach exploits these dynamics to conduct two stimulations in a single ovarian cycle and improve the prognosis of advanced-maternal-age and/or reduced-ovarian-reserve women. Independent groups

worldwide successfully adopted DuoStim with various regimens reporting similar oocyte/embryo competence after both stimulations. Recently, we have demonstrated the fruitful adoption of DuoStim in patients fulfilling the Bologna criteria, especially because of the prevention of treatment discontinuation. Here we aimed at investigating whether DuoStim can be adopted to rescue poor prognosis patients obtaining 0-3 blastocysts after the conventional approach.

Study design, size, duration: Proof-of-concept matched case-control study. All patients obtaining 0-3 blastocysts after conventional-stimulation between 2015-2018 were proposed DuoStim. The 143 couples who accepted were matched for maternal age, sperm factor, cumulus-oocyte-complexes and blastocysts obtained after the first stimulation to 143 couples who did not. The primary outcome was the 1-year cumulative-live-birth-rate. If not delivering, the control group had 1 year to undergo a second attempt with conventional-stimulation. All treatments were concluded (live-birth achieved or no euploid left).

Participants/materials, setting, methods: Only GnRH-antagonist with recombinant-gonadotrophins and agonist trigger stimulation protocols were adopted. All cycles entailed ICSI with ejaculated sperm, blastocyst culture, trophectoderm biopsy, comprehensive-chromosome-testing and vitrified-warmed euploid single-embryo-transfer(s). Cumulative-live-birth-rate was calculated per patient considering both stimulations in the same ovarian cycle (DuoStim group) or up to two stimulations in 1 year (control group). Treatment discontinuation rate in the control group was calculated as patients who did not return for a second stimulation among non-pregnant ones.

Main results and the role of chance: Among the 286 couples included (41.0±2.9yr;4.9±3.1 cumulus-oocytes-complexes and 0.8±0.9 blastocysts), 126 (63 per group), 98 (49 per group), 52 (26 per group) and 10 (5 per group) obtained 0,1,2 and 3 blastocysts after the first stimulation, respectively. The cumulative-live-birth-rate was 9% in the control group after the first attempt (N=13/143). Among the 130 non-pregnant patients, only 12 returned within 1-year (165±95days later;discontinuation rate=118/130,91%), and 3 delivered. Thus, the cumulative-live-birth-rate from two stimulations in 1-year was 11% (N=16/143). In the DuoStim group, the cumulative-live-birth-rate was 24% (N=35/143; Fisher's-exact-test< 0.01,power=80%). The odds-ratio of delivering in the DuoStim versus the control group adjusted for all matching criteria was 3.3,95%CI:1.6-7.0,p<0.01. This difference (0%,22%,15% and 20% in the control versus 10%,31%,46% and 40% in the DuoStim group among patients obtaining 0,1,2 and 3 blastocysts at the first stimulation, respectively) is mainly due to treatment discontinuation in the control group (98%,65%,77% and 80% among patients obtaining 0,1,2 and 3 blastocysts at the first stimulation, respectively) and the further increased maternal age at the time of second retrieval (~6 months). Notably, 2 patients delivered 2 live-births after DuoStim (none in the control) and 14 patients with a live-birth have euploid blastocysts left (2 in the control).

Limitations, reasons for caution: Randomized-controlled-trials and cost-effectiveness analyses are desirable to confirm these data. Moreover, 75% of the patients included were >39yr and 44% obtained no blastocyst after the first stimulation. Therefore future studies among younger women and/or more women obtaining ≥1 blastocyst are advisable to set reasonable cut-off values to apply this strategy.

Wider implications of the findings: A second stimulation in the same ovarian cycle might be envisioned as a rescue strategy for poor IVF outcomes after a first stimulation, so to prevent treatment discontinuation and increase the cumulative-live-birth-rate. This is feasible since 6-7 days span the first and the second stimulation in the DuoStim protocol.

Trial registration number: none

P-607 luteal phase stimulation results in similar euploid blastocysts rate vs. conventional stimulation: donor-recipient cycles

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Study question: Does the luteal phase stimulation (LPS) impact the embryological outcomes, euploid blastocyst rate, and pregnancy rate compared to conventional stimulation in donor-recipient cycles?

Summary answer: LPS is associated with similar embryological outcomes, euploid blastocyst rate, and pregnancy rate among the corresponding recipients compared to stimulation in the follicular phase.

What is known already: LPS has been suggested for fertility preservation in cancer patients, but now it is a part of the new double-stimulation strategy applied to poor responders. Some studies found no difference in the number of oocytes retrieved with LPS compared to conventional stimulation. According to other data, LPS increased numbers of retrieved oocytes compared to follicular stimulation (FPS). Previous studies showed a similar euploid blastocyst formation rate after LPS using the DuoStim approach in poor prognosis patients. However, there is limited data about the embryological outcomes and IVF treatment success in patients with normal ovarian reserve undergoing the LPS.

Study design, size, duration: This prospective observational study included 25 oocyte donors. Group I (n=12) received stimulation on day 2 follicular phase. In group 2 (n=13) received the stimulation on 2-4 day after the ovulation. The definition of spontaneous ovulation included the presence of collapsed follicle/corpus luteum in an ultrasound examination, an increase in the serum P level ≥ 2.0 ng/mL. The blastocyst biopsy and aneuploidy screening were performed for 63 embryos.

Participants/materials, setting, methods: Inclusion criteria: age 18-35 years; basal FSH<10 IU/ml; regular cycle; spontaneous ovulation; AFC>10; normal karyotype; physically and mentally healthy. Exclusion criteria: uterine fibroids; deep endometriosis; PCOS; reduce ovarian reserve. Blastocysts were graded using the Gardner and Schoolcraft classification. Trophectoderm biopsy was performed using the Octax lazer (Sweden). Detection of aneuploidies was performed using the ReproSeq PGS Kit according to the manufactures instruction. Aneuploidy haplotyping was done using Applied Biosystems (4-capillary) Genetic Analyzer.

Main results and the role of chance: No statistically significant differences were found in the number of mature oocytes (20.1±5.58 vs. 21.00±6.1,p=1.0), in the number of donated oocytes (6.17±2.3 vs. 5.7±2.01,p=0.57), in an average number of the blastocyst (4.58 ± 2.2 vs. 3.84 ± 1.8, P = 0.43), in an average number of the euploid blastocyst (1.9 ±1.3 vs. 1.7 ± 1.2, P = 0.78) from FPS versus LPS stimulation, respectively. The euploid blastocyst rate calculated per donated oocytes (30.4% vs. 31.8%,p=1.0), the euploid blastocyst rate calculated per two-pronuclear zygote (31.8% vs. 38%,p=0.56), the euploid blastocyst rate calculated per biopsied blastocyst (63.6% vs. 70%, p=0.78) also were similar after FPS and LPS stimulation. There were no differences between the groups of recipients in fertilization rate (95.7% vs. 83%, p=0.13). Only one embryo was transferred in artificial frozen-thawed embryo transfer to corresponding recipients. The pregnancy rate per embryo transfer was comparable in both groups ((64% (7/11), 95%CI: 30.8–89.0 vs. 62.5% (5/8), 95%CI: 24.5–91.5, p=1.0, in group I and II, respectively).

Limitations, reasons for caution: Our study was carried out in a relatively small subset of patients; therefore, obtained results cannot be extrapolated on other groups of patients and need to be confirmed in larger trials.

Wider implications of the findings: This study opens new possibilities for investigating the luteal. phase stimulation impact on oocyte competence and embryo development

Trial registration number: approved by the ethics committee and Institutional Review Board at 07.09.2017 protocol №10 of Kulakov National Medical Research Centre of Obstetrics, Gynecology and Perinatology. All participants provided written consent.

P-608 The new standard for ovulation triggering should be GnRH agonist rather than hCG during controlled ovarian stimulation for IVF/ICSI: a systematic review and meta-analysis

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Study question: Do Gonadotropin-releasing hormone agonists (GnRHa) triggering improves oocyte maturation, clinical outcomes, and safety compared to human chorionic gonadotropin (hCG) triggering during controlled ovarian stimulation with an antagonist protocol?
Summary answer: The final triggering using GnRHa allows a higher number of retrieved and mature oocytes to be obtained with comparable clinical outcomes and lower OHSS risk.
What is known already: GnRHa represent an alternative to hCG for ovulation triggering after controlled ovarian stimulation with an antagonist protocol for IVF/ICSI. GnRHa triggering is thought to be more physiological due to the endogenous surges in LH and FSH levels. However, the benefit of GnRHa over hCG triggering on oocyte maturation remains controversial.
Study design, size, duration: A systematic review and meta-analysis of randomised controlled clinical trials. Searches were conducted from 01 January 1990 to 15 April 2020 on MEDLINE, EMBASE, the Cochrane Library, ClinicalTrials.gov and EudraCT, using the following search terms: 'GnRH agonist', 'hCG', 'triggering'. Two independent reviewers carried out the study selection, the bias assessment using the RoB2 tool, and the data extraction according to Cochrane methods.
Participants/materials, setting, methods: The primary outcomes were the total number of retrieved oocytes and the number of mature oocytes. The main secondary outcomes were the number of embryos obtained, the clinical pregnancy rate, the early pregnancy loss rate, the live birth rate, and the incidence of ovarian hyperstimulation syndrome (OHSS). Random-effects meta-analysis was performed followed by prespecified sensitivity and subgroup analyses.
Main results and the role of chance: A total of 29 randomised controlled trials were included. The mean number of retrieved oocytes [difference in means (95% CI) 0.99 (0.21, 1.78); p = 0.01; n = 26] and of mature oocytes [0.68 (0.04, 1.33); p = 0.04; n = 12] were statistically significantly higher after GnRHa than after hCG triggering. A similar difference was observed for the number of embryos [0.94 (0.19, 1.68); p = 0.01; n = 10]. No differences in the clinical pregnancy rate [risk ratio 1.01 (0.90, 1.14); p = 0.83; n = 23], early pregnancy loss [1.27 (0.94, 1.71); p = 0.13; n = 16], and live birth rate [1.00 (0.77, 1.29); p = 0.97; n = 6] were noted. GnRHa was associated with a lower incidence of OHSS [odds ratio 0.25 (0.08, 0.74); p = 0.012; n = 20].
Limitations, reasons for caution: The validity of meta-analysis results depends mainly on the quality and the number of the published studies available.
Wider implications of the findings: In light of its safety and effectiveness, GnRHa should be the new standard for triggering in antagonist cycles, with dual triggering with hCG when the risk of OHSS is low and a fresh embryo transfer approach is used.
Trial registration number: NA

P-609 The chances of one live birth rates after first ART cycle in minimal stimulation cycle IVF with letrozole only and natural cycle IVF

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Study question: Are one live birth rates (LBRs) similar in minimal stimulation cycle IVF with letrozole only and natural cycle IVF for the first ART cycle?
Summary answer: LBRs after first ART cycle in minimal stimulation cycle IVF with letrozole only are superior to natural cycle IVF.
What is known already: The addition of letrozole to gonadotropins in ovarian stimulation (OS) may reduce the risk of OHSS, but there is no significant difference were reported in ongoing pregnancy rate or number of oocytes retrieved in the letrozole + FSH group compared to the FSH only. No differences were also reported in clinical pregnancy rates or number of mature oocytes in the additional of letrozole in an GnRH antagonist protocol group compared to the GnRH antagonist group. There are no previous study comparing LBRs after first ART cycle in minimal stimulation cycle IVF with letrozole and natural cycle IVF.
Study design, size, duration: Data for this retrospective cohort study were obtained 643 women, 30-39 years of age started their first ART cycle at one private fertility clinic between January 2016- December 2019.
Participants/materials, setting, methods: A total of 643 women were scheduled their first oocyte retrieval cycle. 118 women started with letrozole (LE) and 525 women started natural cycle (NC). The main strategy for OS in our center is minimal stimulation and natural cycle IVF. Patients consulted with gynecologists to determine their treatment plan based on patients' preference or their menstrual cycle. All pregnancies generated from oocyte retrieval during the first IVF cycle including fresh and frozen-thaw cycles were registered.
Main results and the role of chance: The number of retrieved oocytes and the normal fertilization rates were significantly higher in the LE than NC (4.4 vs 3.4, 77.6% vs 71.1%), p <0.05 respectively). There was no significant difference in the clinical pregnancy rates (CPRs) per embryo transfer (ET) (fresh cleavage stage ET: 32.9% vs 28.0%, frozen-thaw blastocyst ET: 39.4% vs 44.9% ns). However, the CPRs and LBRs per oocyte retrieval (OR) were significantly higher in the LE group (39.0% vs 28.6, 33.9% vs 21.9%, p <0.05 respectively). In a subsequent regression analyses, LBRs per OR of LE was significantly higher than NC as well. (adjusted OR=1.63 (95% CI: 1.02-2.58, p = 0.041).
Limitations, reasons for caution: The strength of the present study was the use of a large cohort of women who underwent minimal stimulation IVF with letrozole only. Although our results are promising, limited by retrospective cohort study. These interpretations prompted the need for a perspective cohort study to evaluate the efficacy of letrozole.
Wider implications of the findings: When comparing minimal stimulation IVF with letrozole only and natural cycle IVF, we found significantly higher LBRs per OR in minimal stimulation IVF with letrozole only, despite similar CPRs per ET.
Trial registration number: none

P-610 Optimal timing of ovulation triggering to achieve highest success rates in natural cycles – an analysis based on follicle size and estradiol concentration in NC-IVF

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Study question: What is the best follicle size, estradiol (E2) serum concentration and endometrial thickness to trigger ovulation in natural cycles?
Summary answer: Optimal follicles size is 18-22mm but estrogen concentration also need to be considered to maximize oocyte maturity and to minimize premature LH surge.
What is known already: Timing of the ovulation triggering is essential in infertility treatments based on natural menstrual cycles such as optimized vaginal intercourse, intrauterine inseminations and thawing cycles without hormone replacement therapy. Common parameters to define the day of ovulation triggering are the follicle size and the estrogen concentration. However, data on follicle size and estrogen concentration are either derived from longitudinal evaluations of few ideal participants, are not very detailed or were studied in stimulated cycles. The model of Natural Cycle IVF (NC-IVF) which provides more detailed information has never been used to study this issue.
Study design, size, duration: Retrospective cross sectional analysis of monofollicular NC-IVF cycles. Follicle size, E2 and LH serum concentrations and endometrial thickness were evaluated on day -5 to 0 (day 0 = day of aspiration). Ovulation was triggered with 5.000IE HCG 36h before aspiration if follicle size was 14-22mm. Patients with irregular cycles, endometriosis >II°, cycles with azoospermia or cryptozoospermia and with inconsistent data were excluded. 606 cycles from 290 women were analysed from 2016 to 2019.
Participants/materials, setting, methods: Mean age of women undergoing NC-IVF was 35.8±4.0y, median 36y [IQ-range: 34;39]. Each woman performed mean 2.1±1.4, median: 2 [IQ-range: 1-3] NC-IVF cycles at an university based IVF center. All parameters were analysed inter and intraindividually and associations were adjusted for maturity of oocyte, zygote development rate, embryo score, implantation rate and live birth rate. Associations were adjusted for age, cause of infertility and number of previous transfers.

Main results and the role of chance: Follicle size, E2 concentration and endometrial thickness increased constantly over time. The increase was computed for each cycle without considering any correlation intra patient, revealing an increase of follicle size by 1.04±0.64mm, an increase of E2 concentration by 167.3±76.8pmol/L and endometrial thickness by 0.69±0.59mm per day.

Based on a multivariate adjusted model with follicle size, E2 and their interaction, number of retrieved oocytes was associated with E2 concentration (aOR 1.80, 95% CI 1.05-3.11; p=0.034). Maturity of oocytes was associated not only with E2 concentration (aOR 1.84, 95% CI 1.15-2.94; p=0.010) but also with follicle size (aOR 1.24, 95% CI 1.01-1.53; p=0.037) and so was also the interaction of both parameters (aOR 0.96, 95% CI 0.94-0.99; p=0.017).

LH surge was calculated to start in 25% of cases at an E2 level of 545 pmol/l, in 50% of cases at 907pmol/l and in 75% of cases at an E2 level of 1531pmol/l.

Live birth rate in cycles with follicles size 14-17 mm was 2.2-3.5% per initiated cycle and in cycles with follicle size 18-22mm 8.5-12.5%.

Limitations, reasons for caution: Cross sectional studies provides less precise information than longitudinal studies. Follicle size and endometrial thickness were evaluated by several physicians possibly causing some imprecision.

Wider implications of the findings: There is a trend towards natural treatment cycles. The study contribute to an optimisation of infertility treatments involving natural cycles. The study gives guidance about the number of days required after a follicle monitoring to reach the optimal time for triggering ovulation.

Trial registration number: not applicable

P-611 Innovative controlled ovarian stimulation (COS) for polycystic ovary syndrome (PCOS) produces high-quality oocytes and no ovarian hyper stimulation syndrome (OHSS)

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Study question: How can we find an ovarian stimulation method that does not cause hyper stimulation syndrome but can produce a high pregnancy rate at one cycle?

Summary answer: This newly developed method for PCOS has a higher accumulative clinical outcome for one trial and no OHSS.

What is known already: Almost all conventional treatments for PCOS have managed to avoid OHSS by reducing the number of growing follicles, which are associated with high Estradiol levels and stimulate the production of vessel endothelial growth hormone (VEGF), leading to increased vessel permeability. Low dose FSH administration, In vitro maturation (IVM), Ovarian Drilling and Coasting have been performed to achieve this. However, their actual clinical outcome is still unsatisfactory.

Study design, size, duration: Evaluation of the efficiency of this method was conducted retrospectively at St. Mother Clinic. The embryonic development and the clinical outcome were studied for 34 PCOS patients during the period between November 2018 and December 2019.

Participants/materials, setting, methods: We started injections of FSH (150iu/ml), then we did ultrasound follicle monitoring. GnRH antagonist shots were started when the leading follicle reached 18mm and continued until the largest follicle was 22-24mm and the E2 value was over 4000pg/ml. Letrozole (2.5mg) and leuprorelin acetate (1.88mg) was injected as trigger. Two tablets each of Letrozole, Cabergoline and GnRH antagonist were given for 5 consecutive days after the oocyte retrieval. All embryos were cryopreserved.

Main results and the role of chance: Number of patients and cycles were 34 and 59. Average number of cryopreserved blastocysts was 6.12 (1-16). The frequencies of OHSS (mild, moderate, severe) were 29.4% (10/34), 0% (0/34), 0% (0/34). Average days between oocyte collection and withdrawal hemorrhage was 5.44(5-7). Cryopreservation rate was 100.0% (34/34). Clinical pregnancy rate and miscarriage rate was 42.3% (25/59) and 16.0% (4/25). The cumulative pregnancy rate was 73.7% (25/34). The four remaining unsuccessful cases still have 10,6,3 and 7 frozen embryos. So, there is a high possibility that they become successful, that would bring the cumulative pregnancy rate up to 82.3% (28/34).

Limitations, reasons for caution: This COS for PCOS seems promising, however it is premature to conclude that this method is established. This method requires caution monitoring for hormone level, follicle size and number and coagulant function. It also accompanied with the risk of ovarian hemorrhage on aspiration of a great number of oocytes.

Wider implications of the findings: This COS seems viable for PCOS cases. It could control the cohort of antral follicles with different doses of Letrozole to find the optimal COH method and it could become the first option for COS of PCOS.

Trial registration number: N/A

P-612 Transdermal testosterone vs. Placebo (lubricant gel) pre-treatment in improving IVF outcomes in diminished ovarian reserve patients (POSEIDON group 3 and 4): a randomised controlled trial

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Study question: To compare the efficacy of transdermal testosterone with placebo (lubricant gel) in improving IVF outcomes using GnRH antagonist protocol in POSEIDON group 3 and 4 patients.

Summary answer: Patients receiving pre-treatment with testosterone gel had higher mean number of oocytes retrieved and grade A embryos as compared to the patients receiving lubricant gel.

What is known already: Diminished ovarian reserve (DOR) is associated with suboptimal ovarian response, higher cycle cancellation rate and lower clinical pregnancy rate following IVF cycles. Various treatment regimens have been devised for management of such patients and use of adjuvants in the form of oral or transdermal androgen is one of them. Androgens improves follicular response to gonadotropin stimulation as well as increase FSH receptor expression in granulosa cells, in turn leading to better oocyte yield and pregnancy rate. Aim was to compare the effect of transdermal testosterone gel with placebo gel on ART outcome in DOR patients (POSEIDON Group 3 and 4).

Study design, size, duration: A prospective, randomised controlled trial was carried out from 1st September 2019 to 31st October 2020 at a tertiary infertility centre in India. 50 patients fulfilling the criteria of Group 3 and Group 4 of POSEIDON classification were included in the study. Patients with endocrine disorders (thyroid, prolactin), endometrioma, history of surgery on the ovaries, sensitivity to testosterone gel, male factor infertility and deranged liver and renal function tests were excluded.

Participants/materials, setting, methods: Enrolled patients were randomised into two groups of 25 patients each, one group was pretreated (TTG group) with transdermal testosterone gel, 12.5 mg/day from day 6th of previous cycle to day 2nd of stimulation cycle while patients in other group took lubricant gel for the same duration before stimulation with GnRH antagonist fixed protocol followed by fresh Day 3 transfer.

Main results and the role of chance: The baseline characteristics of the two groups were comparable. The primary outcome measures were the number of oocytes retrieved and number of grade A embryos formed (according to Istanbul consensus). The secondary outcome measures were implantation rate, clinical pregnancy rate, miscarriage rate and ongoing pregnancy rate. The mean number of oocytes retrieved in TTG group was 5±1.02 which was significantly higher than placebo group-3.5±1.2, (p<0.001). The mean number of Grade A embryos were also significantly higher (4.78±0.54 vs 3.00±0.23, p<0.001) in TTG group. The TTG group had higher implantation rate (28% vs 20%, p= 0.49), clinical pregnancy rate (32% vs 18%, p = 0.41), ongoing pregnancy rate (32% vs 16 %, p= 0.38) and lower miscarriage rate (0% vs 20%, p=0.38), however, these differences were not statistically significant.

Limitations, reasons for caution: The study was done at a single centre with small sample size, replication with more subjects and in different centers is needed.

Wider implications of the findings: Pre-treatment with testosterone gel in DOR patients improves ovarian response to stimulation and results in higher number of oocytes retrieved and good quality embryos resulting in improved clinical pregnancy rates. Transdermal testosterone is advantageous because of better bioavailability, easy application, patient friendly and less adverse effects.

Trial registration number: MCDH/2019/54

P-613 Adjuvant letrozole in ovarian stimulation for in vitro fertilization does not reduce uterine peristalsis frequency prior to fresh embryo transfer

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Study question: Does adjuvant letrozole in ovarian stimulation (OS) for in vitro fertilization (IVF) decrease the uterine peristalsis frequency (UPF) prior to fresh embryo transfer (ET)?
Summary answer: Adjuvant letrozole in (OS) for IVF does not reduce the UPF significantly prior to fresh ET.
What is known already: Throughout the cycle UPF aids spermatozoa transport to the fallopian tube and may affect implantation. At fresh, ET UPF is negatively correlated with implantation- and clinical pregnancy rates and is believed to be modulated by estradiol and progesterone. High levels of estradiol, from multiple follicular development, in OS have been reported to increase UPF, whereas progesterone is considered to be utero-relaxant. The influence of androgens is unclear. Co-treatment with letrozole during gonadotropin OS limits the estradiol rise the supra-physiological estradiol and may therefore reduce UPF prior to fresh ET. Study design, size, duration: This single centre study was nested within a multicentre double blinded RCT investigating the impact of letrozole co-treatment during gonadotropin OS for IVF on late follicular and luteal estradiol, progesterone and testosterone levels. Between 2016 and 2017, 39 women expected normal responders were randomised to co-treatment with letrozole or placebo. Of these, 33 women completed this element of the study. The study was carried out according to the Helsinki Declaration and the ICH-Good-Clinical-Practice.
Participants/materials, setting, methods: Eligible women were randomised 1:1 to adjuvant treatment with letrozole 5 mg/day or placebo in an antagonist protocol using a fixed dose of recFSH 150 IU/day. Final maturation was triggered with rhCG 6,500 IU and luteal support with vaginal progesterone was administered from the day following oocyte aspiration. Less than one hour prior to fresh ET, six minute duration transvaginal ultrasound recordings of the uterus in sagittal section were performed and blood samples were drawn.
Main results and the role of chance: A total of 33 women completed the study (letrozole n=17; placebo n=16). Age, BMI, and ovarian reserve markers were similar between the groups. On day of ET, serum estradiol levels were significantly suppressed in the letrozole group to mean 867 ± 827 pmol/L compared to 3,110 ± 1,528 pmol/L in the placebo group (P < 0.0001). Mean UPF prior to fresh ET did not differ between the intervention and control group (3.3 ± 0.36 versus 3.5 ± 0.51 per minute respectively, P = 0.108). UPF was assessed and agreed by two observers who were blind to adjuvant treatment. Two patients were excluded due to poor quality of the ultra sound recording. Supra-physiological serum estradiol in the placebo group was negatively correlated with UPF (P = 0.014; R = -0.62), but the more physiological serum estradiol levels in the letrozole group showed no correlation with UPF (P = 0.567; R = 0.15). Serum progesterone levels were similar in both groups and did not show any significant correlation with UPF. Testosterone levels were significantly higher in the letrozole group (P = 0.005) and showed a non-significant trend negatively correlated with UPF in the placebo group (P-value=0.07, R= -0.48).
Limitations, reasons for caution: The limited sample size risks masking minor effects.
Wider implications of the findings: The supra-physiological levels of estradiol were significantly supressed in the intervention group, but UPF prior to fresh ET was similar in both groups. UPF is not strongly correlated to luteal phase sex steroid levels. Any beneficial effect of adjuvant letrozole during OS is not through an impact of UPF.
Trial registration number: NCT02939898

P-614 BMI effects on oocyte quality in an egg donation program and the relationship with its first stimulation cycle

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Study question: Does body mass index (BMI) affect oocyte quality in an egg donation program and its relationship with the first stimulation cycle?
Summary answer: Our results indicated significant differences within BMI groups obtaining better results in donors <25 years old in quantity/quality oocyte than > 25 with normal weight.
What is known already: Low weight (LW) and excess weight represent a risk factor for different pathologies and may have a negative effect on the quality of the ovarian response in infertility treatments.
Study design, size, duration: Observational retrospective study at IVI Alicante, in which first donor cycles between 01/01/2015 and 04/30/2020 were analyzed. 307 donors were included. Groups were divided according to the BMI following the WHO criteria and were subdivided according to the protocol used (antagonists / progestogens, age, previous pregnancy and polycystic ovary syndrome (PCOS)). In all cases, the number of total, MII and immature oocytes, doses of FSH and days of stimulation were observed.
Participants/materials, setting, methods: Donors between 18-35 years old, in their first stimulation cycle, were distributed according to their BMI, following the WHO criteria in kg / m2 (Low weight (LW): <18, 5, Normal weight (NW): 18.5-24.9, Overweight (OW) 25-29.9 and Obese (O): ≥30).
Inclusion criteria: Good state of psychophysical health and normal tests according to Spanish law on ART .
Statistical analysis was performed with R statistical software, version 4.0, linear and establishing significant differences when p <0.05.
Main results and the role of chance: Taking into account the general results of the BMI groups by oocytes number and MII: LW: 19.2 / 14.9; NW: 20.2 / 15.6; OW: 18.9 / 14.6, p = 0.513 / p = 0.74 respectively, we observe that with BMI groups and progestin stimulation protocols, results are reversed: No. oocytes and MII with LW: 30 / 22.5; NW: 19.2 / 14.4, OW: 20.8 / 14.8 p = 0.402 / 0.662 respectively. LW and OW are conditions which affect more in quantity than oocyte quality. Significant differences in BMI are observed when they are subdivided according to age, obtaining better results in donors <25 years of age both in quantity and oocyte quality than> 25 years: Total oocytes: LW: 22.6, NW: 21.7, OW: 20 vs LW: 14.2, NW: 17.6, SP: 16.3, p = 0.01, respectively; No. of MII oocytes LW: 17.8, NW: 16.6, OW: 14.9 vs LW: 10.5, NW: 13.9 OW: 13.8, p = 0.003, respectively. Our results reflect that the NW group is the one with better results obtained compared to the LW and OW groups both in overall number of oocytes and also in quality. The type of stimulation does not affect this group of donors.
Limitations, reasons for caution: More studies with clear criteria and a uniform record of results are needed. Meanwhile, the proper shortlisting should be considered if it is a BMI of NW (18.5-24.9 kg / m2) and less than 25 years.
Wider implications of the findings: Analyzing other studies, there are disagreements in terms of discrimination of BMI groups and with incomplete data about of quantity, oocyte quality and oocyte stimulation, offering different results with the same parameters establishing trends in the NW group.
Trial registration number: not applicable

P-615 The effect of late-follicular phase progesterone rise on embryo ploidy, embryo quality and cumulative live birth rates following a freeze-only strategy

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Study question: Is late-follicular phase progesterone elevation (PE) associated with a deleterious effect on embryo euploidy, embryo blastulation and cumulative live birth rates (CLBRs)?
Summary answer: Late-follicular phase PE has no impact on impact on embryo euploidy rate, embryo blastulation rate nor on the CLBR.
What is known already: The effect of PE in ART outcomes has been extensively studied, yielding so far conflicting results. While some authors claim it is only detrimental to endometrial receptivity, others have suggested that it may also impair oocyte/embryo quality. Moreover, little is known regarding the potential effect PE may have on embryo ploidy and, consequently, CLBR.
Study design, size, duration: A multicenter retrospective cross-sectional study was performed between August 2017 and December 2019. A total of 1495 ICSI cycles coupled with preimplantation genetic diagnosis for aneuploidies (PGT-A) and deferred frozen embryo transfer (FET) were analyzed.
Participants/materials, setting, methods: All patients underwent ovarian stimulation with GnRH antagonist protocol and performed a serum progesterone measurement at one of the participating private fertility clinics on the day of trigger. The sample was stratified according to the progesterone levels: normal (≤1.50 ng/ml) and high (>1.50 ng/ml). The primary outcome was the embryo euploidy rate. Secondary outcomes were the number of euploid blastocysts, the blastulation rate and CLBR.
Main results and the role of chance: Late-follicular phase PE was associated with higher late-follicular estradiol levels (2847.56±1091.10 pg/ml vs. 2240.94± 996.37 pg/ml, p<0.001) and more oocytes retrieved (17.67±8.86 vs. 12.70±7.00, p<0.001). The number of euploid embryos was higher in the PE group (2.32±1.74 vs. 1.86±1.42, p<0.001), whereas the embryo euploidy rate (48.3% [44.9%-51.7%] vs. 49.1% [47.7%-50.6%] and blastulation rate (47.1% [43.7%-50.5%] vs. 51.0% [49.7%-52.4%]) were comparable between the two groups. Likewise, no significant differences were found regarding the live birth rate (LBR) after the first FET (34.1% vs. 31.1%, p=0.427) nor the CLBRs (38.9% vs. 37.0%, p=0.637).

Mixed-model analysis was performed in order to account for the clustering of cycles in the same patient. Adjusting for patients' age, PE and BMI, PE failed to demonstrate any effect on the embryo euploidy rate (OR 1.03 [95% CI 0.89-1.20]). Mixed-model analysis for the number of euploid embryos was also performed. After adjusting for PE, age, BMI and ovarian response, PE did not affect the number of euploid embryos (0.02 [95%CI -0.21;0.25]. Multivariate logistic regression adjusted for PE, age, BMI and ovarian response revealed that PE was not associated with the CLBR (adjOR 0.96 [95% CI 0.66-1.38]).
Limitations, reasons for caution: Limitations of the study include its retrospective nature. Moreover, including only GnRH antagonist protocol and ICSI does not allow the extrapolation of these results to other populations.
Wider implications of the findings: Our findings question results from previous studies claiming a detrimental effect of PE on embryo implantation potential. According to our results, PE has no impact on embryo euploidy rate, blastulation rate nor on CLBRs.
Trial registration number: not applicable

P-616 Novel approaches for preventing ovarian hyperstimulation syndrome in breast cancer patients

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Study question: In this study we tried to assess the effectiveness of the use of aromatase inhibitors (AI) for the rapid relief of symptoms of hyperstimulation in patients with breast cancer.
Summary answer: AI showed great efficiency for OHSS prevention and are particulary useful in fertility preservation, when supraphysiologic estradiol levels cause a negative impact and delay treatment.
What is known already: To date the only unequivocally accepted method for fertility preservation is cryopreservation of embryos and unfertilized oocytes. However, controlled ovarian stimulation is associated with supraphysiological serum estradiol level. The majority of guidelines recommend to use aromatase inhibitors during ovarian stimulation (OS) in breast cancer patients with high estrogen receptor expression to protect them from the potential deleterious effects of elevated estrogen. Following oocyte retrieval the patients will be receiving chemotherapy, which is not desirable for hyperstimulated ovaries. The prolonged use of AI seems to be an interesting approach in such cases.
Study design, size, duration: This research was conducted at the V.I. Kulakov NMRC for OG&P to demonstrate management tactics for OHSS prevention after OS in patients with breast cancer. It included 21 patients seeking cryopreservation of oocytes and embryos. The main outcomes included the results of dynamic steroidogeneses assessment, the size of the ovaries, main features of oogenesis and the onset of the menstrual blleding. All patients signed an informed consent form approved by Ethics Committee.
Participants/materials, setting, methods: The mean age of the participants was 26.7. The patients presented prior to gonadotoxic treatment for luminal A or B breast cancer, were randomly divided into two groups of 11 and 10 people and underwent conventional OS with letrozol (2.5 mg/day). Starting from the day of oocyte retrieval the AI dosage iIn the 1st group was increased to 0.75 mg/day; the 2nd group received GnRH antagonist (0.5 mg/day) instead of AI for 5 consecutive days.
Main results and the role of chance: The mean age, BMI and AMH were not different among groups. There was no statistically significant difference in the duration of stimulation and starting and total doses of gonadotropins. The mean number of retrieved oocytes was 15.3 for the 1st group and 16.1 for the 2nd group (p=0.834). There was no significant difference in a number of mature oocytes between the groups (66.1% vs. 72.4% in the 2nd group, p=0.059) or in how many of them formed into 2pn embryos after fertilization (80% vs. 73.9% in the 2nd group, p=0.616). Steroid hormone levels were analyzed during OS and on days 2 and 5 after oocyte retrieval. The rapid decline in serum estradiol and progesterone levels manifested with ovary size reduction and the onset of menstrual bleeding, which were achieved on 3rd to 5th day of AI administration in the 1st group and on 5th to 7th day of GnRH antagonist administration in the 2nd group.
Limitations, reasons for caution: Further research is required to compare the mechanisms of luteolysis induced by aromatase inhibitors and GnRH antagonists to natural luteal regression.
Wider implications of the findings: Our data has demonstrated a greater efficiency of AI compared to GnRH antagonists in reducing the risk of OHSS. These findings could be useful for future research and clinical use in patients without cancer but with a high risk of developing OHSS combined with segmentation of IVF treatment.
Trial registration number: none

P-617 Idiopathic early ovarian aging: Do biomarkers of ageing indicate premenopausal accelerated biological ageing in young women with diminished response to ART?

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Study question: Do young women with idiopathic early ovarian ageing have changes in telomere length and epigenetic age indicating accelerated biological aging?
Summary answer: The telomere length and epigenetic age were comparable to those in young women with normal ovarian ageing.

What is known already: Increased risk of several health events usually considered to be age-related such as cardiovascular disease, osteoporosis, over-all morbidity and mortality have been associated with premature and early menopause when compared to the risk in women with normal menopausal age suggesting an accelerated general ageing process associated to early ovarian ageing. It is unclear whether the onset of this process may start before menopause.
Study design, size, duration: A prospective cohort study. Young women (≤ 37 years) having ART at two Danish Public fertility clinics during the period 2016 to 2018 were divided into two groups dependent on their ovarian reserve status: early ovarian ageing (EOA) (N=55) and normal ovarian ageing (NOA)(N=52). Number of oocytes harvested in first and subsequent cycles was used as a marker of ovarian reserve. Blood samples was drawn at time of oocyte retrieval to assess biological age.
Participants/materials, setting, methods: EOA was defined as ≥ 2 IVF cycles with ≤ 5 harvested oocytes despite sufficient stimulation with FSH and NOA as ≥8 oocytes harvested in minimum 1 cycle. Known causes influencing the ovarian reserve (endometriosis, ovarian surgery, etc.) was reason for exclusion. Relative telomere length (qPCR) and epigenetic age acceleration (DNA methylation levels) were measured in white blood cells as markers of accelerated biological ageing.
Main results and the role of chance: Relative telomere length was comparable with a mean of 0.46 (± sd 0.12) in the EOA group and 0.47 (0.14) in the normal ovarian ageing group (p=0.64). The difference of predicted mean epigenetic age and mean chronological age (i.e. epigenetic age acceleration) was, insignificantly, 0.5 years older in the EOA group when compared to the NOA group((-1.02 years (2.62) and -1.57 years (2.56), respectively, p=0.27)), but this difference disappeared when adjusting for chronological age.
Limitations, reasons for caution: Discrete changes in epigenetic age acceleration may not have been captured as the study only had power to detect an age acceleration of ≥ 2 years.
Wider implications of the findings: By analysis of biomarkers for ageing in whole blood, we did not find any indications of a premenopausal accelerated aging in young women with idiopathic EOA. Further investigations in a similar cohort of premenopausal women is needed to fully elucidate the potential relationship between premenopausal accelerated biological ageing and EOA.
Trial registration number: The study was approved by the Danish Data protection Agency (nr 1-16-02-320-14) and the Regional committee on health research ethics of Central Region Denmark (jr.no 1-10-72-142-14).

P-618 D-Chiro-Inositol and mouse ovary: A new strange case of Dr Jekyll and Mr Hyde?

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Study question: Low doses of D-Chiro-Inositol are beneficial in the treatment of a PCOS mouse model. However, high doses are detrimental for ovarian histology/function. Is D-Chiro-Inositol toxic for the mammalian ovary?
Summary answer: Five mg/day D-Chiro-Inositol for 21 days produced PCOS-like histological/hormonal features. Ten/20 mg/day for 21 days induced ovarian/hormonal states resembling those typical of aged mice.
What is known already: Administration of Myo-Inositol and D-Chiro-Inositol combined according to their plasma molar ratio of 40:1 has beneficial effects in the management of human PCOS. We confirmed the efficacy of this formulation, containing 0.2 mg/day D-Chiro-Inositol, in a mouse model of PCOS. However, formulations containing higher amounts of DCIns had negative effects on ovarian histology and mouse fertility. We investigated possible ovarian toxicity of D-Chiro-Inositol, studying its effects after administration to 30-day-old female mice for 21 days. Young adult mice reproduced the condition of young women possibly facing reproductive/metabolic problems, such as PCOS.
Study design, size, duration: The effects of various doses of D-Chiro-Inositol were analysed on mouse ovarian histology, serum testosterone levels and expression of the ovarian enzyme aromatase. The 21-day period follows normal protocols of pharmacologic PCOS induction in the mouse and spans five ovulatory cycles. Doses employed, 5, 10, 20 mg/day, correspond to doses of 1200, 2400, 4800 mg/day in humans. The first dose is in the range of 1000-1500 mg/day currently prescribed to PCOS patients in clinical practice.
Participants/materials, setting, methods: Five mice/treatment were provided with water administering various doses of D-Chiro-Inositol or 0,5 mg/day letrozole as PCOS-positive controls, for 21 days. At the end of the period, ovulatory cycles were analysed by observations of vaginal cells after vaginal smears; ovarian histology was evaluated by sectioning, hematoxylin-eosin staining and light-transmission microscopy; serum testosterone levels were measured by ELISA; and expression of the ovarian enzyme aromatase was assayed by Western Blots.
Main results and the role of chance: The estrus cycle progressed normally in negative control mice, but was arrested at day 8-10 in the majority of mice under all pharmacologic treatments. Uteri of negative control mice displayed the typical aspect of mature and cycling animals. Uteri of all other mice had an immature/metestrus-diestrus-like aspect, typical of non-cycling animals.

Ovaries of negative control mice showed a normal presence of primary, secondary and tertiary follicles containing a growing oocyte, and of corpora lutea. Ovaries from mice treated with 5 mg/day D-Chiro-Inositol or 0,5 mg/day letrozole had apparently normal primary and secondary follicles but also cystic tertiary follicles resembling those found in human PCOS.

Ovaries from mice treated with 10 or 20 mg/day D-Chiro-Inositol had scarce primary and secondary follicles, a very limited number of tertiary follicles and no cystic follicles, but large follicles/areas with diffused cell proliferation. The typical ovarian structure was lost, especially in the highest dosage.

Treatments with 5 mg/day D-Chiro-Inositol and 0,5 mg/day letrozole increased serum testosterone levels above those of negative control mice, but the former reduced, while the latter increased aromatase levels relative to negative controls. Other treatments had no apparent effects on either testosterone or aromatase levels.

Our experimental paradigm makes the role of chance highly improbable.
Limitations, reasons for caution: The strength of our study relies on the use of an animal model representative of general human tissue organisation and physiological pathways. One weakness consists in the lack of data on serum estrogen levels, due to the paucity of blood provided by a single mouse and the ELISA sensitivity.
Wider implications of the findings: Under all experimental conditions, D-Chiro-Inositol negatively affected ovarian histology and function. Notwithstanding physiological/biochemical differences between mice and humans, caution is therefore recommended when administering D-Chiro-Inositol to PCOS patients at doses corresponding to those we employed in the mouse and/or for long periods, since it may result ineffective or even toxic.
Trial registration number: Not applicable

P-619 Oral dydrogesterone (OD) versus micronized vaginal progesterone (MVP) for luteal phase support (LPS) in IVF/ICSI: a double blind, cross-over, pharmacokinetic study

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Study question: How does the blood pharmacokinetic (PK) profile of OD/MVP differ after the first and last administration dose when used as LPS for fresh embryo transfer? Summary answer: The PK profile differed strongly between both LPS administration strategies with a more rapid absorption, metabolism and clearance of OD in comparison with MVP.
What is known already: Adequate LPS is crucial to achieve a successful pregnancy following ovarian stimulation (OS) and fresh embryo transfer. OD has been proven to be non-inferior compared to MVP in two phase III clinical trials. Additionally, a combined individual participant data and aggregate data meta-analysis showed an odds ratio in favor of OD for live birth. Little information is available on the PK of LPS strategies, leaving an important field unexplored. Individualization of LPS has recently gained more interest and insight into the PK of progestogens is essential to correctly interpret the potential impact of circulating hormone levels on reproductive outcomes.
Study design, size, duration: Twenty oocyte donors underwent two OS cycles followed by one week of LPS (OD or MVP) in a randomized, cross-over,

double blind, double dummy fashion. As both dydrogesterone (D) and 20αdihydrodydrogesterone (DHD) are progestogenic, D, DHD and progesterone (P) plasma levels were established using a validated liquid chromatography tandem mass spectrometry assay in each cycle, on the 1st (single dose PK) and 8th day (multiple dose PK) of LPS (9 and 12 harvesting time-points, respectively).

Participants/materials, setting, methods: All oocyte donors were <35 years, had regular menstrual cycles, no intra-uterine contraceptive device, AMH within normal range and BMI ≤ 29 kg/m2. OS was performed in a GnRH antagonist protocol followed by dual triggering (1000U hCG + 0.2mg triptorelin) as soon as ≥3 follicles of 20mm were present. Following oocyte retrieval, subjects initiated LPS consisting of MVP 200 mg (Utrogestan®) or OD 10 mg (Duphaston®), both three times daily.

Main results and the role of chance: The mean (±SD) age of the subjects was 27.4 (± 3.8) years and BMI was 24.0 (±3.2) kg/m2. The mean (±SD) number of oocytes retrieved was 19.7 ±10. No adverse events were reported during the intake of the study medication. The PK results are best estimates as sampling was reduced compared to a formal PK study. Following the intake of the first dose of OD, the observed maximal plasma concentrations (Cmax) for D and DHD were 2.9 and 77 ng/ml (single dose). The Cmax for D and DHD was reached after 1.5 and 1.6 hours (=Tmax), respectively. On the 8th day of LPS the first administration of that day gave rise to a Cmax of 3.6 and 88 ng/ml for D and DHD (multiple dose). For both, the observed Tmax was 1.5 hours. Following the intake of the first dose of MVP, the Cmax for P was 16 ng/mL with a Tmax of 4.2 hours. On the 8th day of LPS the first administration of that day showed a Cmax for P of 21 ng/mL with a Tmax of 7.3 hours. Although low, the role of chance could be influenced by the relatively low sampling numbers and frequency.

Limitations, reasons for caution: Peripheral concentrations do not necessarily reflect the steroidogenic effect on endometrial progesterone receptors. Extrapolation to clinical practice is therefore difficult, however, molecular analyses of endometrial tissue harvested within this study protocol are underway to investigate further pharmacodynamics and the progestogenic impact on endometrial receptivity during the embryo implantation period.

Wider implications of the findings: This is the first study comparing OD/MVP pharmacokinetics in IVF/ICSI. Results suggest administration frequency to be as important as dose, definitely for OD, showing a rapid absorption/clearance. More studies are needed to investigate blood levels in relation to time of LPS administration, especially in (artificially prepared) FET and LPS individualization.

Trial registration number: EUDRACT 2018-000105-23

P-620 Evaluation of pregnancy and live birth outcomes at the Hewitt Fertility Centre for three newly introduced recombinant gonadotropins when compared to human menopausal gonadotropin

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Study question: To evaluate the effectiveness of three recombinant gonadotropins against the human menopausal gonadotropin currently in use at the fertility clinic.

Summary answer: Human menopausal gonadotropin and Follitropin Delta had the highest pregnancy and live birth outcomes compared to Follitropin Alpha 1 and 2.

What is known already: Currently there is no robust evidence showing a difference in pregnancy and live birth outcomes between urinary (uFSH) and recombinant (rFSH) gonadotropins. However, there is some evidence indicating that uFSH slightly increases pregnancy outcomes in advanced maternal age (AMA). The aim of this study was to evaluate the pregnancy and live birth outcomes in two age groups (<38 years old and ≥38 years old) for three newly introduced rFSH (Follitropin Delta [FD], Follitropin Alpha 1 [FA1], Follitropin Alpha 2 [FA2]) against a human menopausal gonadotropin (hMG).

Study design, size, duration: Patients were randomly allocated to each gonadotropin; hMG, FD, FA1 and FA2. The following outcomes were analysed; positive test rate (PTR) (no. positive test/no. oocyte collections), clinical pregnancy rate (CPR) (no. fetal hearts/no. oocyte collections), biochemical rate (BR) (no. no clinical pregnancies/no. positive tests), implantation rate (IR) (no. sacs seen/no. embryos transferred) and live birth rate (LBR) (no. live births/no. oocyte collection) for two age groups; <38 years old (<38) and ≥38 years old (AMA).

Participants/materials, setting, methods: The study included 1352 patients between July 2018 and December 2019. The <38 group had 1011 patients; hMG (348), FD (38), FA1 (244), FA2 (381). The AMA group had 341 patients; hMG (141), FD (12), FA1 (87), FA2 (101). Chi-square and Kruskal-Wallis tests were used for statistical analysis and p-values of <0.05 were considered statistically significant.

Main results and the role of chance: In the <38 group, hMG and FD had a significantly higher PTR compared to FA1 (32.6%, 43.6%, 22.1% respectively) (p=0.009, p=0.004), as well as a significantly higher CPR (26.9%, 35.9%, 17.5% respectively) (p=0.012, p=0.008) and LBR (21.9%, 33.3%, 13.4% respectively) (p=0.013, p=0.0019). Patients stimulated with FD also had a significantly higher LBR compared to FA2 (33.3%, 19.5%) (p=0.043). No significant differences were seen in the PTR (31%) or CPR (25.1%) when FA2 was compared to hMG and FD. No significant differences were seen between the 4 gonadotropins (hMG, FD, FA1 and FA2) for IR (36.8%, 46.9%, 27.9%, 38.5% respectively) (p=0.14) and BR (17.3%, 17.6%, 21.3%, 19% respectively) (p=0.95)

In the AMA group, hMG, FD and FA1 had a significantly higher CPR compared to FA2 (20.5%, 30%, 20%, 9.3% respectively) (p=0.021, p=0.048, p=0.041). Patients stimulated with FA1 had a significantly higher PTR compared to FA2 (26.3%, 14.4% respectively) (p=0.049). No significant differences were seen compared to hMG and FD (25%, 30% respectively) (p=0.0504, p=0.19). No significant differences were seen between the 4 gonadotropins (hMG, FD, FA1 and FA2) for IR (23.7%, 35.7%, 21.8%, 12.5% respectively) (p=0.094), BR (18.2%, 0%, 23.8%, 33.3% respectively) (p=0.51) and LBR (15.2%, 20%, 12.5%, 9.3% respectively) (p=0.53).

Limitations, reasons for caution: One limitation was that the FD group was the smallest study group; hence further patients should be included to obtain more reliable results. Another limitation was that statistical analysis was not performed using outcomes per cycles started, being unable to know how many abandoned cycles there were for each gonadotropin.

Wider implications of the findings: hMG and FD have had the highest pregnancy and live birth outcomes for both the <38 and AMA groups and the clinic will continue to use both gonadotropins. Parameters such as staff and patient feedback, cost implications and cost-effectiveness per live birth rate now need to be considered.

Trial registration number: NA

P-621 Effects of hormone replacement therapy on osteopenia or osteoporosis in adolescents and young woman with hypogonadism: comparison of oral and transdermal 17 β estradiol administration

Abstract withdrawn by the authors

P-622 Prothrombotic biomarkers during controlled ovarian stimulation for assisted reproductive techniques

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Study question: Does the evolution of prothrombotic biomarkers over time differ between antagonist and long agonist stimulation protocols for assisted reproductive techniques (ART) ?
Summary answer: The hypercoagulable state was higher and persistent in the agonist and antagonist with hCG triggering groups compared to the antagonist with GnRH agonist triggering group.
What is known already: Controlled ovarian stimulation (COS) for ART is associated with supra-physiological serum estradiol levels, a hypercoagulable state and an increased risk of venous thrombosis. Most thromboembolic events associated with COS occur in the context of ovarian hyperstimulation syndrome (OHSS). The use of hCG for final follicular maturation increases the risk of OHSS. In antagonist protocols, GnRH agonist triggering is known to prevent or reduce OHSS and is therefore widely used in women at risk. The impact of the different IVF protocols on pro-thrombotic biomarkers is unknown.
Study design, size, duration: In this prospective observational cohort study, infertile women undergoing COS for ART in 2017-2019 at the University Hospitals of Geneva and Lausanne (Switzerland) were included. We evaluated changes in key coagulation parameters (D-dimers, factor VIII, fibrinogen activity, protein S and protein C) and thrombin generation, our primary outcome, (using 5 pM of tissue factor) by calibrated automated thrombinography before stimulation (T1), on the day of ovulation triggering (T2) and seven days after triggering (T3).
Participants/materials, setting, methods: COS was started without hormonal pre-treatment. Protocols were prescribed according to the standards used in each centre taking into account the risk of OHSS (agonist protocol with hCG trigger in women without OHSS risk (Group 1); antagonist protocol in women at risk of OHSS with hCG trigger (Group 2;) or GnRH agonist trigger (Group 3;); variation of endogenous thrombin potential (ETP) was measured and compared among groups using mixed effects linear regression model.
Main results and the role of chance: A total of 64 women were included: 24 were in group 1, 16 in group 2, and 24 in group 3. The mean age (SD) was 37.8 (2.8), 35.9(5.2) and 34(4.6) years in groups 1, 2 and 3 respectively. As expected, women in group 1 had a statistically lower level of anti-müllerian hormone (p=<0.001), a lower antral follicular count (p=<0.001) and lower number of MII oocytes and embryos obtained (p=<0.001). Mean serum estradiol levels were 1836 (1160), 1628 (815) and 3754 (2165) ng/L at T2, and 945 (471), 1061 (495) and 413 (729) ng/L at T3, in group 1 to 3, respectively. In multivariable regression analysis, the levels in group 3 were statistically higher at T2 and lower at T3 (overall time*group interaction: p<0.001). The mean ETP was similar between all groups at T1, and increased in all groups at T2 (1442, 1426 and 1486 nM/min in groups 1, 2 and 3, respectively) (p=0.013). Overall, ETP evolution over time was statistically different between groups, with the lowest increase of ETP between T1 and T3 in group 3. Protein C and protein S levels were stable, while D-dimers, fibrinogen and factor VIII increased at T2 and T3 in all groups.
Limitations, reasons for caution: Stimulation protocols were prescribed according to the clinical profile and OHSS risks; groups therefore differ substantially in regards to age and ovarian reserve. Thromboembolic events are rare events after COS, we therefore evaluated biological markers of hypercoagulability and not clinical events.
Wider implications of the findings: Women with GnRH agonist triggering protocol did not increase mean ETP in the week after ovulation, while women with hCG triggering did. This different prothrombotic profile was independent of the variation of the other coagulation parameters investigated. This effect of ovulation triggering should be confirmed by further studies.
Trial registration number: NCT04188444

P-623 Comparative preliminary study of LPS, hs CRP and gut bacterial flora of women to support dysbiosis of gut microbiota (DOGMA) as cause of PCOS

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Study question: Study the levels of endotoxin lipopolysaccharide , hs-CRP and gut bacterial flora in Polycystic Ovarian Syndrome to support hypothesis of Dysbiosis of Gut Microbiota (DOGMA) as a cause of PCOS
Summary answer: Increased serum levels of LPS and hs-CRP along with decreased levels of Bifidobacterium and Lactobacilli by quantitative PCR(qPCR) in women with PCOS.
What is known already: A hypothesis called DOGMA was proposed in the "pathogenesis of PCOS" as follows: (1) Obesity, high Carbohydrate , high fat and low fiber diet creat an intestinal flora imbalance, thus break in the intestinal epithelium, thus raising the permeation of the intestinal mucosa; 2) That cause lipopolysaccharide (LPS) to leak into the circulation and the subsequent activation of the immune system may affect the functioning of the insulin receptor resulting in insulin resistance; 3) IR/Hyperinsulinemia can facilitate testosterone synthesis, thus interfering with follicular development.
Study design, size, duration: A descriptive pilot observational study was carried from 1st February 2018 to 31 Dec 2019. The objectives of was to establish and compare basal serum levels of LPS and hs-CRP in 40 women with PCOS (as per modified Rotterdams Criteria) and control group of 40 fertile women and also to determine levels of *Bifidobacterium* and *Lactobacilli* by quantitative PCR (qPCR) in these two groups.
Participants/materials, setting, methods: A total of 80 consenting women underwent sonographic , laparoscopic and hormonal evaluation and were grouped into 40 with PCOS as per Rotterdams criteria and 40 healthy control group. The blood samples were taken for estimating hsCRP and LPS. The stool samples were also collected in transport medium and refrigerated . The qPCR was run on stool sample to determine levels of Bifidobacterium and Lactobacillus. The results obtained are compared and presented.
Main results and the role of chance: The mean age, weight, height and BMI in PCOS (Grp 1) was comparable with controls group(Grp 2). Annovulation (33/40) was more prevalent in PCOS group along with bulky ovaries with raised ovarian volume (Rt 12.86±2.25 mm, / Lt 12.77±1.43 mm.) . The mean serum

FSH (4.51±0.95 / 4.80±0.81) were comparable between groups.The serum LH (10.56±1.28/ 5.18±1.19), AMH (7.72±1.62/ than group 2/ 4.77±1.10) and serum testosterone levels (39.39±7.47/20.46±3.09), were found to be increased in Grp 1 The hs-CRP (2.68±0.61/ 1.40±0.52)and LPS levels 14.95±1.97/ 11.35±1.63) were significantly increased in Grp 1 ,while abundance of *Bifidobacterium*, and *lactobacillus (* 4.39±0.80 & 4.33±1.11) in the control group as compared with the PCOS group (2.10±0.51 and 2.20±0.65) was also noted. The raised hs-CRP (a non specific marker of inflammation), raised endotoxin Lipopolysaccharide (secreted by Gram negative bacteria which leak and react with receptors may cause inflammation in PCOS), and quantitative decreased bacterial load of *Bifidobacterium*, and *lactobacillus* as estimated by quantitative PCR in infertile women with PCOS and that of fertile women support dysbiosis as cause of PCOS.

Limitations, reasons for caution: The study was carried out on population with traditional high prevelance of diorrheal and other infective diseases which might confound the results given ibid. Addition of treatment arm with probiotics y a large multicentric study will give further insight to the dysbiosis and PCOS

Wider implications of the findings: The study elaborates the effect of dysbiosis as cause of PCOS and gives direction for alternative strategies like probiotics in management of PCOS.

Trial registration number: Not Applicable

P-624 Doxorubicin exposure detrimentally effects establishment of early ovarian reserve, ex-vivo mouse model

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Study question: Does exposure to chemotherapy agents used to treat cancer during pregnancy cause significant decrease in early establishment of the ovarian reserve?

Summary answer: Significant oocyte loss is noted following exposure of early mouse ovaries to doxorubicin. This apoptotic decline is not seen with cisplatin, docetaxel or paclitaxel exposure.

What is known already: Chemotherapy has been shown to adversely affect the ovarian reserve of pre-pubertal and reproductive age females. However, little is known regarding the effects in the next generation following treatment in pregnancy beyond observational population studies that examined rates of birth defects and developmental delays. Reproductive function in offspring develops over time and would be difficult to quantify in these short-term studies. An ex-vivo mouse ovary culture offers the ability to closely mimic maternal treatment level serum concentrations and directly evaluate the impact on oocyte number and cell death markers.

Study design, size, duration: In mice, the ovarian reserve is matured postnatally, mimicking the biologic second trimester activity of the human ovary. Wild-type C57bl/6 pups were collected on postnatal day 0. Ovaries were cultured in hanging well organ culture media with addition of DMSO or a chemotherapy agent. Immunofluorescence was used to quantify oocyte number and density. Power calculation showed a N of 11 per drug would be needed to demonstrate a decrease of ⅔ or more. Participants/materials, setting, methods: 83 ovaries were cultured, sectioned and analyzed in duplicate. Planned analysis at serum max and mid concentrations was performed at 48 hours and 5 days. Given clinically variation in concentrations of cisplatin, additional concentration samples were added. After noting the degradation following exposure to doxorubicin, additional samples at earlier time points of 12 and 24 hours of exposure were added for dynamic evaluation. Means were calculated and then compared using a 2-way ANOVA.

Main results and the role of chance: Doxorubicin exposure during establishment of the ovarian reserve resulted in a significant loss of oocyte number and density. At 12 hours a 22% decrease was noted; this increased to a loss of 91% of oocytes by 48 hours of exposure. The oocyte density fell from 693 oocytes/ mm² in the control to only 63 oocytes/ mm² in the serum max concentration (p=0.003). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) revealed early impact within the stroma by 12 hours with widespread apoptotic changes by 24 hours.

Treatment with cisplatin resulted in a phenotypic change in the oocyte population with preservation of smaller, more peripheral cells. The average oocyte density remained similar to control even at the highest clinical concentrations, 536 oocytes/ mm² compared to 570 oocytes/ mm² (p=0.772). Docetaxel and paclitaxel demonstrated an increase in oocyte number and density, though not enough to reach significance. The oocyte density 5 days following docetaxel exposure was 802 oocytes/ mm², a 41% increase (p= 0.12). The oocyte density 5 days following paclitaxel exposure was 780 oocytes/ mm², a 37% increase (p=0.817). The drug exposure did impact stromal cells, as noted in TUNEL images.

Limitations, reasons for caution: This ex-vivo mouse model offers tight control of chemotherapy concentration, it does not account for filtration and modification by the placenta. Longer term cultures may also demonstrate that temporary arrest in small oocytes, such as those exposed to cisplatin, do not thrive and later progress to apoptosis.

Wider implications of the findings: Doxorubicin is employed as the most common chemotherapy during pregnancy. Utilization may be to the serious detriment of the younger generation. If other alternatives are clinically effective they should be considered. No other models have explored the effect of fetal ovary exposure to chemotherapy on the establishment of ovarian reserve.

Trial registration number: not applicable

P-625 Does endogenous progesterone play a role in unexplained infertility? A systematic review

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Study question: Does endogenous progesterone play a role in unexplained infertility? A systematic review investigating the possibility of altered progesterone-mediated change leading to reduced endometrial receptivity in women with unexplained infertility.

Summary answer: The evidence suggests that many of the measurable actions of endogenous progesterone are reduced in women with unexplained infertility when compared with controls.

What is known already: Unexplained infertility is the diagnosis given to heterosexual couples who fail to conceive despite normal semen analysis, regular ovulation and patent tubes. The underlying pathology is likely to relate to embryonic failure to implant. Endometrial receptivity is largely mediated by luteal phase progesterone which controls many different molecular pathways involved in secretory transformation. It is possible that defective actions of progesterone could contribute to this condition. To date however, there is minimal published literature on the role of progesterone in unexplained infertility. We therefore felt it important to combine the results of all trials measuring progesterone-related outcomes in unexplained infertility.

Study design, size, duration: A systematic review was performed using standard Cochrane methodology. We searched Medline, Embase and CINAHL databases from inception to December 2020 and additionally hand-searched. The study was prospectively registered on Prospero (CRD42020141041). The search strategy was designed to identify all types of primary research published in English that investigated women with unexplained infertility and reported outcomes that relate to progesterone. Newcastle Ottawa Scoring and NHLBI assessment of bias scoring was performed.

Participants/materials, setting, methods: The study population was women with unexplained infertility. Included studies had no controls, fertile controls or controls with other diagnoses associated with subfertility. Outcomes were either upstream affecting progesterone production/release or receptor expression or downstream measuring results of progesterone-mediated processes. The results were summarised in a narrative review. Meta-analysis was not possible due to varying methodological heterogeneity.

Main results and the role of chance: 36 studies were included. No difference was found in 18 studies in progesterone levels (serum, peritoneal and salivary) between women with unexplained infertility and control groups. Despite

this, 32 of the 36 included studies demonstrated a significant difference between progesterone-mediated outcomes in the unexplained infertile and control groups.

5 ultrasound studies all reported increased resistance and decreased flow on doppler studies of uterine, ovarian and spiral arteries and reduced endometrial and sub-endometrial perfusion. No significant difference was found in luteal phase endometrial thickness in 2 studies.

Endometrial dating was reported by 11 studies. 8/11 studies reported significantly higher numbers (20-38%) of 'out-of-phase' endometrium in women with unexplained infertility compared with controls.

Endometrial biopsy results measuring different cell adhesion molecules, monoclonal antibodies and other molecules involved in endometrial transformation as well as expression of responsible genes and steroid hormone receptors were included. All the progesterone-mediated outcome measures listed above were reduced in unexplained infertile women except β3 integrin which reported contradictory results and SGK1 expression which was reported in 1 study. This trend towards support for the hypothesis may be more important than any individual finding. The quality of the included studies was variable and hence the strength of the recommendations moderate.

Limitations, reasons for caution: The number of studies measuring each outcome was limited. The study quality varied from good to poor. Methodological heterogeneity between studies prevented meta-analysis. The strength of the study however comes from the originality of the research, the variety of included outcomes and that 32/36 papers reported results supporting the hypothesis.

Wider implications of the findings: The findings of this systematic review support the need for larger, well designed research on this topic. If altered progesterone-mediated receptivity is implicated in unexplained infertility, it may be possible to offer other therapeutic interventions to improve outcomes as an alternative or adjunct to standard fertility treatment.

Trial registration number: NA

P-626 A freeze-all strategy improves clinical pregnancy rate in patients with few available embryos

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Study question: Is elective frozen blastocyst transfer an advantageous strategy for all patients?

Summary answer: A freeze-all strategy improves the outcomes in patients with few available embryos.

What is known already: With the aim of defining the best moment to perform embryo transfer, in recent years, relevance has been given to the understanding of the implantation window, however oocyte and embryo quality are key factors that are not to be disregarded.

It has been suggested that a freeze-all strategy and subsequent frozen embryo transfers improve pregnancy rates. However, it is unclear whether this strategy benefits all kind of patients (i.e. with or without surplus embryos, etc).

In this study, we aim to provide an answer on which patients may benefit of a freeze-all policy and a subsequent frozen embryo transfer.

Study design, size, duration: This retrospective cohort study includes infertile patients aged 21 to 44 years old, without previous history of recurrent failure of ART (including recurrent miscarriages). Enrolments took place between January 2015 and November 2019 and cycles with oocyte donation and PGT were excluded. Embryo transfers were performed in: 1) a fresh cycle (ET) or 2) a deferred cycle with surplus frozen embryos (FET) or embryos that were frozen in a freeze-all policy (FET-FA).

Participants/materials, setting, methods: Patients with blastocysts transfer were included. PGT cycles were excluded.

The number of cycles complying with the inclusion criteria were: 617 ICSI cycles. Fresh embryo transfers (ET) were performed in 396 cycles (43 with a subsequent Frozen embryo transfer, FET). Frozen embryo transfers following a freeze-all strategy (FET-FA) were performed in 221 cycles.

Clinical pregnancy rates (CPR) and Cumulative clinical pregnancy rates (CCPR) were calculated and compared among those groups.

Main results and the role of chance: Mean age of patients was 36.1 ± 3.7 years old (mean ± SD). In average, 1.83 ± 0.41 (mean ± SD) embryos were transferred.

Following the first transfer (either ET and FET-FA), CPR was 40.4% and 58.4% (ET and FET-FA, respectively). Following the subset analysis of 2 age groups (≤38 & >38 years-old); in the ≤38-group, CPR was 45.2% and 58.9% (ET and FET-FA, respectively), while in >38-group, the rates were 30.8% and 54.8% (ET and FET-FA, respectively); $p<0.05$. CCPR were also significantly better in the FET-FA group: 51.3% vs 66.8% and 33.8% vs 58.1% in the ≤38-group and >38-group, respectively.

Additionally, CPR was analysed independently for patients with ≤2 usable embryos (1 attempt) or ≥3 usable embryos (surplus embryos after first attempt).

When a single attempt was possible; in the ≤38-group, CPR was 36.1% and 56.9% (ET and FET-FA, respectively) while in the >38-group, the rates were 24.7% and 63.6% (ET and FET-FA, respectively); $p<0.05$. When surplus embryos were available, no difference in CPR (or CCPR) between ET and FET-FA groups were observed. After first attempt CPR were 58.4% and 48.2% in the ≤38-group & >38-group, respectively; while CCPR were 69.8% and 57.1% in the ≤38-group & >38-group, respectively.

Limitations, reasons for caution: Although the authors consider that the patient population is of optimal size, a detailed analysis of the stimulation protocol and hormonal values (estradiol and progesterone) during treatment, and its potential relation to the outcomes, should follow.

Wider implications of the findings: In our setting, the data suggests that freeze-all strategy (with subsequent frozen embryo transfer) over fresh transfer is advantageous for patients with few available embryos (1 or 2 embryos for a single attempt). This increases the chances to pregnancy in 30.3% in the ≤38-group and 77.9% in the >38-group.

Trial registration number: Not applicable

P-627 Optimal timing of day 6 blastocyst transfer in artificially prepared frozen-thawed embryo transfer cycles

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Study question: When is the optimal timing of day 6 (D6) blastocyst transfer between the 6thday (P6)and the 7th(P7) day of progesterone administration in artificially prepared frozen-thawed embryo transfer(FET) cycle

Summary answer: When transferring D6 blastocysts in artificially prepared FET cycles, live birth rate tended to be higher in P6 group than in P7 group.

What is known already: Blastocyst transfer in FET cycles has increased due to several reasons including convenience for optimization of endometrial synchronization, improvement of laboratory techniques and preimplantation genetic testing. Meanwhile, D6 blastocyst which cryopreserved on day 6 after being developed to the full blastocyst stage, presented lower pregnancy outcomes in FET cycle than D5 blastocysts. However, there have been few studies on the optimal duration of progesterone administration when transferring D6 blastocysts.

Study design, size, duration: This was a retrospective cohort study including patients who underwent frozen-thawed blastocyst transfer in artificially prepared cycles from January 2000 to May 2020. Patients with D6 blastocyst transfer on the 6th day of progesterone administration were included in D6-P6 group, and patients with D6 blastocyst transfer on the 7th day of progesterone administration were included in D6-P7 group.

Participants/materials, setting, methods: Increasing dose of estradiol valerate was administered from the 3rd day of menstruation: 4 mg/day for the first four days, 6 mg/day for next four days, and then 8 mg/day until the confirmation of pregnancy. Progesterone was administered from the 14th day of menstruation if the endometrial thickness reached ≥7 mm. The independent t-test or Mann-Whitney test, chi-square test, and logistic regression analysis were performed.

Main results and the role of chance: A total of 50 patients were included, and 13 patients underwent FET on P6 and 37 patients underwent FET on P7. Live birth rate was comparable between the P6 group and the P7 group (18.9% vs. 15.4%, $p=0.775$). Live birth rate was higher in the D6-P6 group than in the D6-P7 group after adjusting for age, AMH, endometrial thickness on the starting day of progesterone administration and good embryo rate transferred with statistical significance (OR: 6.716, $p=0.005$).

Limitations, reasons for caution: Limitations of the present study is the retrospective design and the small sample size. Caution is needed in extrapolating results of this study because only intramural and vaginal progesterone supplementations were included in this study.
Wider implications of the findings: Even if the duration of blastocyst formation was delayed, frozen-thawed D6 blastocyst may need to be considered for on P6 rather than P7. The difference of live birth rate is not statistically significant. This study should be acknowledged for the underestimation of the difference because of the small sample size.
Trial registration number: not applicable

P-628 Predicting oocyte yield in patients with diminished ovarian reserve using basal antral follicle count

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Study question: Is basal antral follicle count (bAFC) taken on day 1 to 3 of stimulation a useful predictor of oocyte yield in that cycle, in women with diminished ovarian reserve (DOR)?
Summary answer: Basal AFC has moderate correlation with final oocyte yield. A median 75% of the antral follicle count is collected as oocytes.
What is known already: The current theory of folliculogenesis suggests that all follicles available for recruitment are visible on ultrasound in the ovary at the point when ovarian stimulation is applied. This implies a tight correlation between the AFC on day 1-3 of a stimulation cycle (bAFC) and the eventual number of follicles collected. We hypothesise that in women with diminished ovarian reserve who receive maximum stimulation basal AFC would be a useful predictor of final oocyte yield in that cycle.
Study design, size, duration: This was a prospective single centre, observational study in a tertiary referral hospital in London. 125 women with DOR underwent controlled ovarian stimulation between December 2018 and January 2021.
Participants/materials, setting, methods: All study participants were given an antagonist cycle with a starting stimulation dose of 450iu and remained on the same dose throughout their treatment. We assessed the correlation between bAFC taken on day 1-3 of the stimulation cycle and the total number of oocytes collected.
Main results and the role of chance: A total of 150 treatment cycles were included in the analysis. The median age was 37 (IQR 35 – 39). The median AMH was 6.0 (IQR 4.4 – 8.9) and the median FSH was 7.6 (IQR 5.7 – 9.4). The median bAFC at the start was 9 (IQR 6 – 11). The median total stimulation dose was 4050iu (IQR 4050 – 4500). The median oestradiol on day of trigger was 5906 (IQR 4166 – 7397) and median number of oocytes collected was 7 (IQR 5 – 9).

There was a moderate correlation between bAFC and the number of oocytes collected (r= 0.549, p=0.005). The median ratio of oocytes collected over the number of antral follicles observed at the start was 72.7% (IQR 58.3 – 100).
Limitations, reasons for caution: We have standardised approach to AFC determination and have previously shown that AFC inter and intra-observer variability in our unit is low. Nevertheless, our study involved multiple operators for AFC determination which may introduce variability. Further variability may have been introduced at egg collection by varying technique.
Wider implications of the findings: Studies of antagonist protocol in good prognosis patients suggest poor correlation between basal AFC and oocyte yield. In contrast, our study shows that in a population of women with DOR basal AFC provides useful information which can be used to counsel women around the expected oocyte yield of their cycle.
Trial registration number: not applicable

P-629 Is spontaneous ovulation better than induced ovulation for frozen-thawed embryo transfer cycle?

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Study question: Between spontaneous ovulation (SPO) and induced ovulation (INO) comparing clinical pregnancy rate and ongoing pregnancy rate for frozen-thawed embryo transfer (FET) cycle, which is better?
Summary answer: Both spontaneous ovulation and induced ovulation protocols showed no significant difference in clinical pregnancy rates and ongoing pregnancy rates.
What is known already: Recent practice worldwide is moving towards elective freezing of all embryos and subsequent frozen-thawed transfer, both for a perceived higher pregnancy rate as well as the significant reduction of ovarian hyperstimulation. The timing of FET can be determined by either detecting the spontaneous Luteinizing Hormone surge (SPO group) or by the administration of hCG (INO group). There is still an ongoing debate to determine which is the best protocol for frozen-thawed embryo transfer in the non-hormone replacement therapy (non-HRT) cycle.
Study design, size, duration: This retrospective study included 500 FET cycles for patients who had regular menses between June 2017 and June 2020. The FET cycles were grouped by type as follows: SPO (n = 281) and INO (n = 219). The primary outcome was the clinical pregnancy rate and the secondary outcome was ongoing pregnancy rate. Ongoing pregnancy is defined as a viable intrauterine pregnancy at 12 weeks of gestation confirmed on an ultrasound scan.
Participants/materials, setting, methods: This study was conducted in a single IVF centre. Vitrification was used as the cryopreservation method. To standardize outcome measures, only patients having single blastocyst transfer and aged under 38 years old were included. The average age of the patient was 32.9. Gamete donation, embryo donation, pre-implantation testing and assisted hatching cycles were also excluded from the analysis. Categorical data were analysed using Chi-square test SPSS version 25.
Main results and the role of chance: Clinical pregnancy rate for SPO group was 54.8% (154/281) versus 52.9% (116/219) in INO group. Even though clinical pregnancy rate was higher in SPO group as compared to INO group, it did not reach significance level (2 = 0.17, p = 0.68). As all patients had single blastocyst transferred, the implantation rate was the same as clinical pregnancy rate. Ongoing pregnancy rate was also found higher in SPO group as compared to INO group (135/281, 48.0% and 97/219, 44.3% respectively) but again failed to reach significance level (2 = 0.70, p = 0.40).
Limitations, reasons for caution: The retrospective nature of the study and therefore, the analysis was not adjusted for confounding factors such as blastocyst grading, etiology of infertility, and ethnicity of patients.
Wider implications of the findings: In natural cycle, both spontaneous ovulation and induced ovulation protocols had the same pregnancy outcomes for frozen-thawed embryo transfer. However, induced ovulation can facilitate in scheduling FET timing to avoid weekends and public holidays, if necessary.
Trial registration number: Not applicable

P-630 Progesterone levels using pessaries of 400 mg of vaginal progesterone (Cyclogest®) in artificial cycles for frozen embryo transfer

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Study question: Does the use of pessaries of 400 mg of micronized progesterone provide comparable results as pessaries of 200 mg x2, in terms of progesterone levels?
Summary answer: The administration of pessaries of Cyclogest® 400 mg reduces the probability of presenting suboptimal level of progesterone on the day of the embryo transfer.
What is known already: The endometrial preparation for frozen embryo transfer (FET) in Artificial Cycle (AC) with vaginally-administered progesterone, is one of the most common IVF procedures nowadays. Now, it has been shown that suboptimal progesterone levels on the day of the embryo transfer compromise the results of FET treatments. Recently, a new preparation of 400 mg vaginal pessaries has been introduced in the market of European countries. Efficacy of this new preparation has been studied in "fresh" IVF cycles but we lack the comparative studies in AC making it necessary to further investigate this area.
Study design, size, duration: Non-inferiority retrospective case-control trial based on 347 embryo transfer treatments with endometrial preparation in AC

carried out at Instituto Bernabeu between January 2019 and July 2020. 153 patients received 1 pessary of 400 mg every 12 hours (group A) and 194 received 2 pessaries of 200 mg every 12 hours (group B). Sampled size calculation resulted in 182 patients required to detect a minimum difference of 2 ng/ml so sample was powered for the purpose.

Participants/materials, setting, methods: Patients receiving embryos in AC preparation were included. All embryo transfers were performed at blastocyst stage after 5 days of progesterone administration. Progesterone levels were assessed the day of the embryo transfer by an electrochemiluminescence immunoassay.Primary outcome was the incidence of suboptimal progesterone levels according with the cutoff value stablished in the literature at 8.8 ng/mL. Secondary outcomes were pregnancy rates (PR), clinical pregnancy rates (CPR), ongoing pregnancy rates (OPR) and miscarriage rates (MR).

Main results and the role of chance: Incidence of suboptimal levels of progesterone was significantly lower in the group of 400 mg (9,8% in Group A vs 19,7% in the Group B, p=0.011). Given that there was an imbalance between groups in the body weight (66.9 +/- 14 vs. 61.9 +/- 13.165 kg, p<0.001) and BMI (24.63 +/- 4.861 vs. 22.54 +/- 3.092, p<0.001), we decided to perform a binary logistic regression setting patient's weight and BMI as confounding variables. The result confirms a higher risk of suboptimal progesterone levels (<8.8) with the 2x200 mg regimen (OR: 2.52 95%CI: 1.28-4.96; p=0.007). Mean progesterone levels were similar in both groups (13,8035 ng/mL +/- 4.62159 vs. 13.9799 ng/mL +/- 7.73243 respectively, p=0.146). No differences were observed in clinical outcomes: PR (52.3% vs. 53.1%, p=0.881), BM (14.7 % vs. 17.6 %, p=0.597), CM (20% vs. 18.6 %, p=0.819) and OPR (33.1 % vs. 33.7 %, p=0.912). The subjective medical decision to administer additional progesterone from the day of the embryo transfer onwards (taking values other than 8.8 ng / mL as a reference), was significantly lower in the group of 400 mg (24,3% vs 37,3%, p=0.009).

Limitations, reasons for caution: The inherent limitations of a retrospective analysis. The study was not powered to detect differences in clinical outcomes. Therefore, results other than progesterone levels should be interpreted with caution.

Wider implications of the findings: A single pessary of 400 mg minimizes the necessity of additional medication (usually subcutaneous progesterone). Presentation of 400 mg is superior to 2x200 providing adequate progesterone levels and patient comfort. Dose finding and pharmacokinetics studies of the vaginal administration will be necessary for the future to optimize FET under AC.

Trial registration number: NCT04722471

P-631 Embryo euploidy rates following follicular or luteal start ovarian stimulation. A prospective study with repeated ovarian stimulation ovarian stimulation cycles

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Study question: Is there any difference in embryo euploidy rates following luteal phase phase (LS) and follicular phase (FS) start ovarian stimulation. Summary answer: The number of euploid blastocysts and embryo euploidy rate are comparable when comparing FS and LS.

What is known already: Random start ovarian stimulation (starting at any time of the cycle) has been traditionally used in women undergoing urgent fertility preservation for medical reason. Although there is accumulating evidence that in infertile women, LS can result in equivalent number of oocytes and embryos as compared with FS, no study has evaluated the effect of luteal phase start ovarian stimulation on embryo euploidy rates. The current study is the first prospective study designed to evaluate embryo euploidy rates in donors undergoing two identical consecutive ovarian stimulation protocols within a period of 6 months starting either in the (FS), or (LS).

Study design, size, duration: In a prospective study, conducted between May 2018 and January 2020, 40 oocyte donors underwent two consecutive ovarian stimulation protocols within a period of 6 months with an identical fixed GnRH antagonist protocol starting either in the early follicular (FS), or and luteal menstrual cycle phase (LS).

Participants/materials, setting, methods: All participants underwent two identical consecutive ovarian stimulation cycles with 150µg corifollitropin alfa followed by 200 IU rFSH in a fixed GnRH antagonist protocol either in the FS or LS. Six MII oocytes from the same oocyte donor, from each stimulation cycle, were allocated to the recipients and were inseminated with the same sperm sample (recipients partner sperm or donor sperm). Embryos were cultivated to blastocyst stage followed by preimplantation genetic testing for aneuploidies (PGT-A).

Main results and the role of chance: When comparing FP with LP, the duration of ovarian stimulation was significantly shorter (9.68± 2.09 vs 10.93± 1.55 days), 95% CI [-1.95; -0.55] and a higher total additional dose of daily recFSH was significantly lower (526.14± 338.94 IU vs 726.14± 366.27), 95% CI [-315,12; -84,88] when CPT was administered in the luteal phase. . There were no differences in the hormone values on the triggering day (Estradiol 2137.61±1198.25 pg/ml vs 2362.96±1472.89); 95% CI [-1160.45;709.76]. Overall no differences were observed in the number of oocytes (24.84± 11.200 vs 24.27± 9.08); 95% CI[-2,61; 3.75] and MII oocytes (21.41±10.19 vs 21.59± 8.81), 95%CI [-2.72; 2.35] retrieved between FP and LP cycles in the oocytes donors. Following oocyte allocation and fertilization to the recipients, a total of 245 blastocysts were biopsied (blastocyst formation rate 245/408, 60.05%), 117 in FP group and 128 in LP group. The overall blastocyst euploidy rate was 59.18% . There were no differences in the number of euploid embryos between FS (1.59±1.32) and LS (1.70±1.29), mean difference 0.11, 95%CI [-0.65; 0.46]. Finally, there were no differences in the percentage of euploid embryos per oocytes inseminated between FS [70/287 (24.4%)] and LP [75/278 (24.7%), mean difference -0.027, 95%CI [-0.11; 0.06].

Limitations, reasons for caution: The study was performed in oocyte derived from potentially fertile young oocyte donors thus caution is needed when extrapolating the results in oocytes derived from infertile women of older age.

Wider implications of the findings: Luteal phase stimulation does not alter embryo euploidy status as compared with follicular phase stimulation and thus it appears that it can be safely used not only in cases of urgent medical fertility preservation but also in patients undergoing ovarian stimulation for IVF/ICSI.

Trial registration number: Clinical Trials Gov (NCT03555942).

P-632 Examination of temporal changes in phenotype and gut microbiome during the process of growth in polycystic ovary syndrome (PCOS) model induced by prenatal androgen exposure

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Study question: From when do abnormality in gut microbiome and phenotypes of PCOS appear during the process of growth?

Summary answer: Reproductive phenotypes of PCOS appear from 6 weeks and metabolic phenotypes from 12 weeks onward. Alteration in gut microbiome appears as early as 4 weeks.

What is known already: The etiology of PCOS remains largely unknown, however PCOS is considered as a complex multigenic disorder with strong epigenetic and environmental influence. Previous studies have suggested that fetal over-exposure to androgens could be the main factor of the development of PCOS after birth. On the other hands, recent studies on both human and PCOS rodent models have demonstrated the association between PCOS and alteration of gut microbiome in adulthood. Furthermore, it was recently reported that gut microbiome in obese adolescent with PCOS is different from obese adolescent without PCOS.

Study design, size, duration: A rodent PCOS model induced by prenatal dehydroepiandrosterone (DHT) exposure was applied to this study. Phenotypes and gut microbiome were compared between PCOS model mice (n=12/group) and control mice (n=10/group) at each stage of growth; 4 weeks (prepuberty), 6 weeks (puberty), 8 weeks (adolescent), 12 weeks (young adult), and 16 weeks (adult). The determinants for PCOS phenotypes are onset of puberty, estrous cycle, morphology of ovaries, serum testosterone level, body weight, and insulin resistance.

Participants/materials, setting, methods: Pregnant dams were subcutaneously injected on days of 16, 17, and 18 of gestation with either sesame oil for control groups or sesame oil containing 250µg of DHT for prenatal DHT groups. The evaluation of PCOS phenotypes and gut microbiome in female

offspring were performed at each stage of growth. For examination of gut microbiota, next generation sequencing and bioinformatics analysis of 16S rRNA genes were performed on DNA extracted from mouse fecal samples.
Main results and the role of chance: Prenatal DHT mice exhibited delayed puberty onset, disrupted estrous cycle, and significantly increased testosterone levels from 6 weeks onward. Significantly increased atretic antral follicles were observed in prenatal DHT mice at 6, 12, and 16 weeks. Prenatal DHT mice showed significantly decreased body weight at 4, 6, 8 weeks and increased body weight from 12 weeks onward. As for gut microbiome, alpha-diversity was significantly different between control and prenatal DHT mice from 8 weeks onward and beta-diversity was significantly different at 6 and 8 weeks. Altered composition of gut microbiota was observed as early as 4 weeks. At phylum level, Firmicutes are significantly increased in prenatal DHT mice at 4 and 8 weeks and decreased at 16 weeks. Actinobacteria phylum showed significant decrease at 6 and 8 weeks in prenatal DHT mice. At genus level, relative abundance of several bacterial taxa significantly differed between control and prenatal DHT mice; some taxa, such as Allobaculum, Adlercreutzia, Biloph ila, Clostridium, Gemella, Gemmiger, Roseburia, Ruminococcus, Staphylococcus, and Sutterella, exhibited constant increase or decrease in prenatal DHT mice during the process of growth. Interestingly, Roseburia was never detected in prenatal DHT mice, while approximately half of control mice harbored Roseburia at 12 and 16 weeks.
Limitations, reasons for caution: It is not clearly determined whether alteration in gut microbiome is cause or result of PCOS development, although the changes in gut microbiome seemed to precede the appearance of typical PCOS phenotypes in the present study. Mouse model does not completely recapitulate human PCOS.
Wider implications of the findings: Our findings suggest that prenatal androgen exposure causes alteration of gut microbiome from pre-puberty onward, even before PCOS phenotypes become apparent. Intervention for girls at risk of PCOS with pre/pro-biotics may prevent them from developing PCOS in future.
Trial registration number: Not applicable

P-633 lifestyle intervention prior to IVF does not improve embryo utilization rate and cumulative live birth rate in women with obesity

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Study question: Does lifestyle intervention prior to in vitro fertilization (IVF) improve embryo utilization rate (EUR) and cumulative live birth rate (CLBR) in women with obesity?
Summary answer: A six-month lifestyle intervention preceding IVF improved neither EUR, nor CLBR in women with obesity.
What is known already: A randomized controlled trial (RCT) evaluating the efficacy of a low caloric liquid formula diet (LCD) preceding IVF in women with obesity was unable to demonstrate an effect of LCD on embryo quality and live birth rate. In that study, only one fresh embryo transfer (ET) or, in case of freeze-all strategy, the first transfer with frozen-thawed embryos was reported. We hypothesized that any effect on embryo quality of a lifestyle intervention in women with obesity undergoing IVF treatment is better revealed by EUR and CLBR after transfer of fresh and frozen-thawed embryos.
Study design, size, duration: This is a nested cohort study within an RCT. The LIFEstyle study examined whether a six-month lifestyle intervention prior to assisted reproductive technology (ART) in women with obesity improved live birth rate, compared to prompt ART within 24 months after randomization. In the original study, 577 women with obesity and infertility were assigned to a lifestyle intervention followed by ART (N = 290) or to prompt ART (N = 287) between 2009 and 2012.
Participants/materials, setting, methods: The first IVF cycle with successful oocyte retrieval was included, resulting in 51 participants in the intervention group and 72 in the control group. EUR was defined as the proportion of inseminated/injected oocytes that could be transferred or cryopreserved as an embryo. Analysis was performed per cycle and per oocyte/embryo. CLBR was defined as the percentage of participants with at least one live birth from the first fresh and subsequent frozen-thawed ET(s).
Main results and the role of chance: The overall mean age was 31.64 years, and the mean BMI was 35.40 ± 3.21 kg/m2 in the intervention group, and 34.86 ± 2.86 kg/m2 in the control group (P = 0.33). The mean difference of weight change at six months between the two groups was in favor of the intervention group (mean difference in kg: -3.14, 95% CI: -5.73 – -0.56). The median (Q25; Q75) of EUR was 33.3% (12.5; 60.0) in the intervention group and 33.3% (16.7; 50.0) in the control group in the per cycle analysis (adjusted B: 2.7%, 95% CI: -8.6 – 14.0). In the per oocyte/embryo analysis, in total 280 oocytes were injected or inseminated in the intervention group, 113 were utilized (transferred or cryopreserved embryos, EUR = 40.4%); in the control group EUR was 30.8% (142/461). The lifestyle intervention did not significantly improve EUR (adjusted OR: 1.36, 95% CI: 0.94 – 1.98) in the per oocyte/embryo analysis taking into account the interdependency of the oocytes per participant. CLBR was not significantly different between the intervention group and the control group after adjusting for type of infertility (male factor and unexplained) and smoking (27.5% vs 22.2%, adjusted OR: 1.03, 95% CI: 0.43 – 2.47).
Limitations, reasons for caution: This study is a nested cohort study within an RCT, and no power calculation was performed. The randomization was not stratified for indicated treatment. The limited absolute weight loss and the short duration of the lifestyle intervention might be insufficient to affect EUR and CLBR.
Wider implications of the findings: Our data do not support the hypothesis of a beneficial effect of lifestyle intervention on embryo quality and CLBR after IVF in women with obesity.
Trial registration number: NTR 1530

P-634 The effect of bariatric surgery on PCOS patients' obstetrical and neonatal outcomes: A population based study

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Study question: Does bariatric surgery (BS) decrease the occurrence of adverse obstetrical and neonatal outcomes in morbidly obese women with polycystic ovarian syndrome (PCOS)?
Summary answer: Patients with PCOS who underwent BS were at lower risk for gestational diabetes mellitus (GDM) and pregnancy-induced hypertension (PIH), than other women with PCOS.
What is known already: Studies have shown that PCOS in pregnancy is associated with the occurrence of GDM, preeclampsia, PIH, preterm birth (PTB), cesarean section, miscarriage, hypoglycemia, and neonatal death. Patients with PCOS may have an increased risk of obesity compared to the general population, which magnifies the inherent insulin resistance many fold. PCOS patients who underwent bariatric surgery have a marked improvement in menstrual irregularities, hirsutism, T2DM, hypertension, and dyslipidemia. The benefit of bariatric surgery for PCOS patients and the improvement of pregnancy related complications has not been adequately studied, with most studies being small.
Study design, size, duration: This is a retrospective study using the Health Care Cost and Utilization Project-Nationwide Inpatient Sample (HCUP-NIS) database from 2004 to 2014. Using multivariate logistic regression analysis, we compared women with PCOS who underwent BS with a control group consisting

of pregnant patients with PCOS of all BMIs who had not had weight reduction operations regarding pregnancy, delivery, and neonatal outcomes.
Participants/materials, setting, methods: The study group included pregnant PCOS patients who underwent BS and the control group consisted of pregnant patients with PCOS; who delivered between 2004 and 2014. Demographic and clinical characteristics, pregnancy, delivery, and neonatal outcomes were compared. Multivariate logistic regression analysis was performed to control for all statistically different (P<0.05) confounding effects. Each subject was included once per delivery.
Main results and the role of chance: We identified 9,096,788 pregnancies during the study period. 141 patients had a history of PCOS and underwent BS. The control group was composed of 14,741 patients with PCOS who didn't undergo BS. Prevalence of PCOS patients who underwent BS increased from 0/1,000 in 2004 to 14.6/1,000 in 2014(p=0.001). Those who underwent BS were more likely to be older than 35-years(42.6% vs. 18.7%, p<0.0001), obese at delivery(44.7% vs. 22%, p<0.0001) and have a history of previous cesarean sections (24.8% vs. 18.2%, p=0.045). Differences in pre-gestational diabetes (7.1% vs. 4.1%, p=0.086) and hypertension (12% vs. 8.4%, p=0.125) .The BS group was less likely to develop PIH (aOR-0.39, 95%CI-0.21-0.72) and GDM(aOR-0.40, 95%CI-0.23-0.70) when compared to the control group. When comparing the PCOS with and without BS; differences in gestational hypertension(95%CI-0.22-1.30), preeclampsia(95%CI-0.19-1.15), spontaneous vaginal delivery (95%CI 0.58-1.3), operative vaginal delivery (95%CI 0.34-1.8), Cesarean section (95%CI 0.79-1.79), post partum hemorrhage (95%CI-0.12-1.94), transfusion (95%CI-0.1-5.22), preterm delivery (95%CI-0.56-1.75) and maternal infection (95%CI-0.27-2.07) were similar. Regarding neonatal outcomes of PCOS with and without BS: small for gestational age babies (95%CI-0.26-2.68), and congenital anomalies (95%CI-0.09-4.52) were similar.
Limitations, reasons for caution: Limitations of our study include its retrospective design. Information regarding the time interval between the surgical intervention and pregnancy wasn't available. Nor was information on the type of gastric bypass performed.
Wider implications of the findings: Our study demonstrated an association between bariatric surgery in the setting of PCOS patients and a reduced risk of GDM and PIH. Although no differences were noted in other delivery and neonatal outcomes, data was not compared to a group of strictly obese PCOS controls.
Trial registration number: not applicable

P-635 Standard versus mild ovarian stimulation in women with polycystic ovaries (PCO): Impact on outcomes in subsequent frozen embryo treatment cycles (FET)

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Study question: Does mild ovarian stimulation in women with PCO result in higher live birth rates during subsequent FET cycles?
Summary answer: Mild ovarian stimulation with FSH doses <150IU did not result in higher clinical pregnancy or livebirth rates in subsequent FET.
What is known already: Ovarian stimulation during IVF in women with PCO is associated with an exaggerated response, ovarian hyperstimulation syndrome, poor egg to follicle ratio, low fertilisation rates and poor blastocyst conversion. Mild ovarian stimulation, often referred to as protocols with FSH doses under 150IU, is often employed to overcome these challenges. One of the perceived benefits of this approach is improved oocyte and embryo quality reflected in lower aneuploidy rates.
Study design, size, duration: This was a retrospective observational study looking at 99 FET between January 2011 and Jan 2021 that followed a fresh cycle in women with a pre-treatment antral follicle count of 12+12 or greater. Patients were identified through the antral follicle count at the pre-treatment investigation ultrasound scan. Ultrasound findings, treatment cycle details and clinical outcomes were entered prospectively into a dedicated clinic database. Data was retrieved and analysed using SPSS V25.
Participants/materials, setting, methods: The study was conducted in a large IVF centre. Data on women with an AFC of 12+12 or above, undergoing an autologous FET cycle following a fresh cycle were collected. Women were split into those receiving <150IU of FSH (Group1, n= 51) and those receiving FSH ³150 IU (Group 2, n=48). Binary logistic regression analysis was performed to control for confounders. Live birth was the primary outcome, with biochemical and clinical pregnancy being secondary outcomes.
Main results and the role of chance: Women in Group 1 were younger (30.8±3.6 v 33.8±3.65, p<0.005) but had a similar antral follicle count (38.2±11.7 v 34.2±9.1, p=0.07). The total number of eggs collected (24.1±13.8 v 25.9±8.8, p=0.45) and fertilisation rate (0.59±0.2 v 0.58±0.18, p=0.77) during their fresh cycle were comparable. Women in Group 2 had a larger number of embryos suitable for cryopreservation (7.36±4.2 v 4.8±3.5, p=0.001)

In the subsequent frozen embryo replacement cycle, there was no difference in the number or quality of embryos transferred with most women having a single embryo transfer (63% v 48%, p=0.14) and at least one top quality embryo transferred (68.6% v 81%, p=0.15). There was a higher biochemical pregnancy rate in Group 1 (84% v 66%, p=0.035) but with no difference in clinical pregnancy rate (53% v 44%, p=0.37) or live birth rate (49% v 42%, p=0.76). Live birth rates remained comparable even after controlling for age, and number and quality of embryos transferred (OR: 1.21 (95% CI 0.50-2.94).
Limitations, reasons for caution: This was a retrospective analysis raising the risk of allocation bias. This study was also at risk of information bias as it relied on accurate documentation of the AFC at the pre-treatment scan.
Wider implications of the findings: Patients can be reassured that both stimulation protocols result in similar live birth rates in subsequent frozen embryo replacement cycles.

Prospective trials using PGT-A are required to assess whether aneuploidy could account for the discrepancy in biochemical pregnancy rates in the two groups considering the subsequent comparable clinical pregnancy rates.
Trial registration number: Not Applicable

P-636 Anti-Müllerian-Hormone levels are not correlated to the probability of obtaining an ongoing pregnancy and time to pregnancy in women undergoing intra-uterine inseminations with donor sperm

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Study question: Is Anti-Müllerian hormone (AMH) level associated with the probability of obtaining an ongoing pregnancy (OP) and with time to pregnancy (TTP) in women undergoing d-IUI?
Summary answer: AMH is neither associated with the probability of obtaining an OP nor with time to pregnancy (TTP) in women undergoing d-IUI
What is known already: Anti-Müllerian hormone (AMH) is a glycoprotein produced by the granulosa cells of preantral and antral follicles. While AMH has been widely recognized as a quantitative marker of ovarian reserve used to predict ovarian responsiveness to ovarian stimulation in IVF, its relationship with fecundability in spontaneous conceptions is still a matter of debate. There is currently no consensus on the role of AMH on time to pregnancy in unassisted conceptions. The question of whether AMH is a qualitative marker of oocyte quality is therefore still unanswered.
Study design, size, duration: This prospective cohort study was carried out between 9/1/2017 and 12/30/2020 on 592 women aged 19 to 44, who underwent d-IUI in a natural cycle (n=1788) the day after LH peak. Patients were single, homosexual, or heterosexual with an infertile partner and underwent 1 to maximum 6 d-IUI. All patients had regular ovulatory cycles and bilateral tubal patency confirmed before starting d-IUI. AMH evaluation was performed within the previous 3 months of the first d-IUI.
Participants/materials, setting, methods: The primary outcomes were the likelihood of obtaining an OP (>14 weeks) and TTP calculated as the number of d-IIU up to an OP. Multivariate logistic regression was used to compare the probability of obtaining an OP according to age and AMH levels. Kaplan-Meier

curves with log-rank test were used to assess the TTP stratified by age groups (≤35, >35 to ≤39, and > 39 years old) and AMH groups (< 1ng/mL and ≥ 1ng/mL).
Main results and the role of chance: AMH levels were negatively correlated with age (p<0.001). OP were significantly lower with increasing age (OR 0.92 (0.89-0.95) p<0.001) but did not differ according to AMH levels (OR 1.07 (0.97-1.18) p=0.18). When adjusting for AMH, the association between age and OP remained significant (OR 0.91 (0.88-0.95)). TTP was significantly different between age groups: ≤35 years old (n=338), >35 to ≤39 years old (n=136) and > 39 years old (n=118) (p<0.001), but did not differ significantly according to AMH levels < 1ng/mL (n=130) and ≥1ng/mL (n=462)(p=0.55).
Limitations, reasons for caution: Our results concern d-IUI and can therefore not be extrapolated to natural conception. However, the study model is close to natural fecundity as there was no history of female infertility and as all IUI were performed on the day of ovulation with donor sperm proven to be fertile.
Wider implications of the findings: While measuring AMH seems necessary for gonadotropin dose adjustment in ART, our data suggest that it cannot qualitatively assess fertility outcome and should therefore not be used routinely for preconception counseling in the absence of infertility history.
Trial registration number: P2017/396

P-637 Development and validation of an Artificial Intelligence algorithm that matches a clinician ability to select the best follitropin dose for ovarian stimulation

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Study question: Is it possible for an Artificial Intelligence (AI) model to match the performance of clinicians in prescribing the first dose of follitropin?
Summary answer: The AI based Decision Support System (DSS) we developed identifies accurately the optimal starting dose range of follitropin and prospectively matches the clinicians' performance.
What is known already: Most patients treated by IVF undergo Controlled Ovarian Stimulation (COS). Based on their ovarian markers, demographic characteristics, and clinical history, an initial dose of follitropin is prescribed. Failing to tailor correctly this dose can result in a suboptimal ovarian response, leading on the one hand to low and ineffective response or, on the other, to excessive and dangerous stimulation. AI methods can learn from large databases of COS results and generate predictive models to assist the clinicians in optimizing this decision.
Study design, size, duration: A database of 2713 first IVF cycles from 5 clinics, from 2011 to 2019 was used to develop the model. Predictor variables included: age, BMI, AMH, FSH, LH, estradiol, Antral Follicular Count (AFC), infertility etiology, and previous live births. 80% of the database was used to train the algorithm, and 20% to test the DSS. Additional 524 cycles from a different period (2020-2021) were used for prospective validation.
Participants/materials, setting, methods: Follitropin dosage was divided in 4 categories: 100-150IU, 151-200IU, 201-250IU, and >250IU. An optimal ovarian response is defined as retrieving 10-15 MII, whenever the patient ovarian reserve allows it. To predict the optimal dose range personalized to each patient, the DSS uses a Random Forest model learned with training cycles. To evaluate the DSS performance, a score for each dose range and each patient was defined given the prescribed doses and the corresponding ovarian responses.
Main results and the role of chance: The cycles included in the database were from women 37.2±4.9 years old [18-45], with a BMI of 23.7±4.2, AMH of 2.4±2.3, AFC of 11.8±7.7; the average number of oocytes and MII obtained was 10.1±7.1 and 7.2±5.3, respectively. The DSS achieved a performance mean score of 0.88 in the testing database, a value significantly better than the one calculated for the doses prescribed by the clinicians, which had a mean score of 0.83 (p-value <0.05). In the validation database the mean performance score of the DSS recommendations was 0.87, and there were no significant differences with the score of the doses actually prescribed by clinicians, also with a score of 0.86. With these results the model was shown to at least match the performance of the human doctor. It is worthy of note that the performance score value for the doses prescribed by clinicians in the validation database is relevantly higher than in the test database, closing the gap previously existing with the DSS performance. As the validation cycles are separated temporally from the rest of the cases and correspond to the newer ones, it is plausible to infer that a more experienced clinical staff would perform better.
Limitations, reasons for caution: The DSS prospective validation should be extended to more clinical cases to ensure higher reliability. Hyper-responders were underrepresented in the database which can lead to less accurate recommendation in some of these women. As all AI models, the DSS should be tested prospectively before clinical application.
Wider implications of the findings: The AI based clinical Decision Support System that we developed could be deployed as a training and learning tool for new clinicians and serve as quality control for experienced ones; further, it can be used as an electronic second opinion, for instance by providing information in peer-to-peer case discussions.
Trial registration number: not applicable

P-638 Status of insulin resistance in infertile women and its effect on ovulation induction

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Study question: Is insulin resistance (IR) a confounding variable in infertile women, other than those those having polycystic ovarian syndrome (PCOS)
Summary answer: IR was identified in 20.5% of infertile women. The presence of IR did not affect response to ovulation induction but reduced chances of conception
What is known already: Obesity is strongly correlated with insulin resistance. Obesity also has an adverse effect on fertility. In 2008 Steeg et al reported 5% reduction in chances of spontaneous conception with each unit increase in body mass index (BMI). *Tetsurou Sakumoto et al (2010) reported* hyperinsulinemia to affect granulose cells in small follicles inducing early response to luteinising hormone and anovulation. Adverse effect on endometrial function and implantation was also postulated. Insulin resistance has been studied in cases of PCOS but has not been studied in infertile women not fulfilling criteria for diagnosis of PCOS . So the present study was planned
Study design, size, duration: A prospective cohort study was conducted in infertility unit, King George Medical University, Lucknow, India over a period of one year from August 2018 to July 2019. Total 102 women with unexplained infertility were enrolled. Ethical clearance was obtained from institutional ethical committee
Participants/materials, setting, methods: Women with PCOS; diminished ovarian reserve documented by antral follicle count <7 and anti Mullerian hormone < 1.1ng/ml; bilateral tubal block; abnormal semen analysis; untreated hypothyroidism, hyperprolactinaemia; known diabetes were excluded. All women underwent ovulation induction with clomiphene citrate followed by single intrauterine insemination. Homeostasis model assessment insulin resistance index (HOMA IR) was calculated

HOMA-IR = Fasting S. Glucose (mg/dl) x Fasting insulin (μIU) / 405.

Value ≥ 2 denoted insulin resistance

Main results and the role of chance: : IR was identified in 21/102 (20.5%) cases. Fasting insulin levels were in the range of 5 – 9.9 mIU/ml in 53/102 women; <5mIU/ml in 29 and >10mIU/ml in 20. Fasting insulin > 9.45mIU/ml was found to have 90.5% sensitivity and 96.3% specificity in predicting insulin resistance. None of the cases had abnormal fasting and post prandial plasma glucose levels. IR was seen to be significantly correlated with BMI > 25kg/m2 (p=0.0018) and waist hip ratio of > 0.85 (p=0.0024).All women had follicular development and follicle rupture irrespective of presence of IR. Women with IR were more likely to have monofollicular development (17/21 IR cases). Correlation of endometrial thickness with IR was not seen. Mean endometrial thickness was 8.9mm. There were 6 pregnancies among the 102 women studied. None of the women with IR conceived.

So IR was found to be affecting one fifth of women with unexplained infertility. Failure of any woman with IR to conceive was significant but the finding needs to be further studied.
Limitations, reasons for caution: : It was a small study with only 102 cases and the women were followed for only one cycle of ovulation induction and

intrauterine insemination so results need to be validated in a larger study with a longer follow up.
Wider implications of the findings: If further larger studies corroborate the role of IR in women with unexplained infertility it could elucidate the possibility of using insulin sensitisers in management of such cases. IR may emerge as an important gamechanger in management of unexplained infertility.
Trial registration number: not applicable

P-639 The role of chronic inflammation in polycystic ovarian syndrome – a systematic review and meta-analysis

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Study question: Is polycystic ovarian syndrome (PCOS) associated with chronic inflammation as determined by elevated serum C-reactive protein (CRP) level independent of obesity? Summary answer: Circulating CRP is moderately elevated in women with PCOS (independent of obesity), which is indicative of low-grade chronic inflammation.
What is known already: Although current literature associates polycystic ovarian syndrome (PCOS) with chronic inflammation, the evidence for this link remains inconclusive and its causal nature remains unclear. A systematic review and meta-analysis involving 31 studies was published on this topic in 2011 providing evidence for increased circulating CRP (96% higher than controls). However, since that review there have been over 100 published studies assessing CRP in PCOS women utilising more advanced CRP assays.
Study design, size, duration: This systematic review involved an extensive search of electronic databases for studies investigating CRP and other inflammatory makers in PCOS women from January 2000 to March 2020. Searched databases included PUBMED, EMBASE and MEDLINE, SCOPUS, DynaMed plus, TRIP, ScienceDirect and Cochrane Library. Inclusion criteria were using Rotterdam criteria for PCOS diagnosis, measuring CRP with high-sensitivity assay, matching/adjusting participants for BMI, and including drug naïve participants who were free from conditions that could affect inflammatory markers.
Participants/materials, setting, methods: The review included all studies comparing circulating CRP between women with and without PCOS. Articles' quality and risk of bias were assessed using modified Newcastle-Ottawa scale. CRP data were extracted from eligible studies and entered into RevMan software for calculation of standardized mean difference (SMD) and 95% Confidence Interval (CI). Sensitive analysis was performed for high-quality studies providing data for non-obese participants.
Main results and the role of chance: The systematic review included 95 eligible studies (n=10,074), of which 68 (n=7991) were included in a meta-analysis. Sixty-two of the 95 studies reported significantly higher circulating CRP in PCOS women (n=5235) versus controls (n=4839). The remaining studies showed no statistically significant differences between the two groups after adjusting for BMI. Pooled analysis of 68 studies revealed significantly higher circulating CRP in PCOS women (SMD 1.26, 95%CI, 1.01, 1.52; z=9.60; p=0.00001; I^2=96%). Sensitivity meta-analysis for non-obese women in 37 high-quality studies showed significantly higher circulating CRP in PCOS women versus controls (SMD 1.84, 95%CI, 1.40, 2.28; z=8.19; p<0.00001; I^2=97%). Circulating TNF-α was measured in 13 studies, of which seven reported higher levels in PCOS women versus controls and six showed no difference. Circulating IL-6 was measured in 19 articles, of which eight reported significantly higher levels in PCOS women versus controls and 11 found no difference. Four studies (n=512) reported increased white cell count in PCOS women (n=323) compared with healthy controls (n=189).

Nine studies (n= 922) assessed circulating adiponectin, with seven showing significantly lower levels in PCOS women (n=368) versus controls and one showing no difference. Meta-analysis of four of these studies (n=355) revealed a SMD -1.48 (95% CI; -2.48,-.14).
Limitations, reasons for caution: High heterogeneity between studies and the small size of several studies are the main limitations. Heterogeneity is due to variation in laboratory methods used to measure CRP and variations between participants e.g. age, BMI and PCOS phenotypes. Sensitivity and sub-group analysis were performed to address this heterogeneity.
Wider implications of the findings: Further research is required to understand the underlying molecular mechanisms and the pathophysiological role of chronic inflammation in PCOS. This could potentially identify targets for new treatments that could improve short- and long-term health problems associated with PCOS.
Trial registration number: N/A

P-640 High physical activity and ovarian reserve: A prospective study of normo-ovulatory professional athletes

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Study question: Is high physical activity (HPA) associated with low ovarian reserve in normo-ovulatory, reproductive-age women?
Summary answer: HPA does not affect ovarian reserve negatively.
What is known already: HPA is associated with menstrual irregularities and subsequent potential infertility, probably through hypothalamic neuroendocrine pathways. However, it is not yet known whether HPA influences the ovarian reserves of normo-ovulatory, reproductive-age women.
Study design, size, duration: This observational, cross-sectional study compared 30 professional female athletes who were engaged in HPA for at least 3 years prior to study recruitment, with high International Physical Activity Questionnaire (IPAQ) scores and 30 women who did not engage in physical activity. The study was conducted at a tertiary medical center from 2017-2020.
Participants/materials, setting, methods: Physically active, normo-ovulatory women (n = 30), ages 20-35 years were recruited from The Wingate Institute, the Israeli National Institute for Sport Excellence. Non-physically active women (n=30), matched by age and BMI to the HPA group, were recruited from the hospital staff. Both groups were evaluated for ovarian reserve markers on day 2-5 of the menstrual cycle, including follicular stimulating hormone (FSH), antral follicle count (AFC), anti-Mullerian hormone (AMH) and Inhibin B.
Main results and the role of chance: The average age of the athletes (HPA group) was 30.1±2.1 years and of the nonactive (control) group 31.6±3.8 years (p=0.071). BMI of the 2 groups was similar (22.6±2.4 vs. 21.3±2.6; p=0.075) for the HPA and control groups, respectively. Regarding ovarian reserve, no significant differences were observed between the HPA group and the control group with respect to FSH (p=0.304), AFC (p=0.27), AMH (0.507) or Inhibin B (p=0.074). For the HPA group, older age at menarche was positively associated with AFC (p=0.008) and AMH (p=0.009) and not with FSH levels (p=0.313). For the nonactive group, no significant association between age at menarche and FSH levels, AFC or AMH was found (p=0.433, p=0.274 and p=0.163, respectively). Additionally, for the HPA group, duration of physical activity per week (hours) was not significantly associated with FSH levels, AFC or AMH (p=0.619, p=0.608 or p=0.997, respectively).
Limitations, reasons for caution: Although we investigated the ovarian reserves of 30 women engaged in HPA, a larger cohort would provide more information. Information on diet and sleep habits was not evaluated and may result in some confounding. Moreover, it would be more informative if we also followed these women regarding fecundability and fertility.
Wider implications of the findings: This study demonstrated that HPA may not negatively affect ovarian reserve markers. These findings may provide reassurance for women who are engaged in HPA and attempting pregnancy. Further research needs s to be confuted.
Trial registration number: 0247-16

P-641 Pubertal high-fat diet interferes with the ovarian kisspeptin ligand and receptor expressions regulating female fertility

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Study question: Does chronic high-fat diet affect ovarian dysfunctions via changing of kisspeptin and kisspeptin receptor expressions?
Summary answer: Ovarian kisspeptin and kisspeptin receptor expressions are significantly affected by the chronic high-fat diet.
What is known already: Regarding the hypothalamic-pituitary-gonadal (HPG) roles of kisspeptin, it appears that it directly stimulates LH secretion from the pituitary, consecutively stimulates ovulation in the female. There are also studies showing that kisspeptins can increase GnRH release, serum FSH, LH, and testosterone (in vivo), and regulate ovulation in women who have reached sexual maturity through the central control of the HPG axis.

Fatty acids can act as nutritional signals that regulate the HPG axis, and elevated levels of circulating saturated fatty acids associated with high-fat diet (HFD)-feeding has been shown to induce ovarian dysfunction. HFD consumption induces ovarian dysfunction in rodents.
Study design, size, duration: 4-week-old female rats obtained from Akdeniz University Experimental Animals Unit. Animals were kept in standard conditions; fed with control diet consisting of standard laboratory food (13.5% of total energy from oil) or HFD (60% of total energy from oil) for 8 weeks. Experimental procedures were performed at the age of 12 weeks (250-275 g live weight). Live weights and food consumption of the animals in both groups were calculated weekly and recorded during the experiment.
Participants/materials, setting, methods: At the end of the experimental period, the animals were sacrificed and tissues were obtained. Sections were taken from paraffin-embedded tissues. Immunohistochemical staining for KISS1 and GPR54 were performed in both groups' ovaries. Tissue collecting, processing, and immunohistochemical staining were performed at the histology and embryology department of Akdeniz University Faculty of Medicine.
Main results and the role of chance: There was a significant increase in body weights of the HFD group during the experiment period compared to the control group.

As a result of immunohistochemical staining, kisspeptin expression is specifically localized to the corpora lutea of the control ovaries. However, kisspeptin expression in the corpora lutea of the HFD ovaries was increased regardless of the ovarian follicles.

KISS1R expression was located in the cytoplasm of oocytes. HFD group also expressed the KISS1R in the oocytes with increasing intensity.
Limitations, reasons for caution: The study design included pubertal age limit of the samples. The functional reproductive period in different mammal species should be considered.
Wider implications of the findings: We suggest that chronic exposure of female rats to a high-fat diet may induce ovarian Kisspeptin expression while kisspeptin receptor expression is not affected in oocytes. Clinical implications should be considered.
Trial registration number: B.30.2.AKD.0.05.07.00/99

P-642 Does Conn's syndrome (Primary hyper-aldosteronism) affect pregnancy and neonatal outcomes? A population based study of over 9 million deliveries.

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Study question: Does primary hyperaldosteronism(PA) confer an independent risk for adverse pregnancy or neonatal outcomes, based on analysis of the Healthcare-Cost and Utilization Project-Nationwide Inpatient Sample(HCUP-NIS) database?
Summary answer: After controlling for all significant confounders, women with PA are at increased risk for gestational hypertension, eclampsia, and operative vaginal delivery, but unexpectedly not preeclampsia
What is known already: PA is extremely rare in pregnancy, and our current knowledge regarding PA during pregnancy is derived only from case reports and series. No pregnancy control studies exist in the literature for this endocrinopathy. PA is characterized by autonomous aldosterone and suppressed rennin production from the adrenals. Caused by adenomas or hyperplasia, it presents with hypertension and hypokalaemia.
Study design, size, duration: This is a retrospective population-based cohort study utilizing data from the HCUP-NIS from 2004 to 2014, inclusively. A cohort of all deliveries during the study period was created. Within this group, all deliveries to women with PA were identified as part of the study group (n=102), and the remaining deliveries were categorized as the reference group (n=9,096,686).
Participants/materials, setting, methods: HCUP-NIS is the largest inpatient sample database in the USA and is comprised of hospital stays throughout the country. It provides information relating to seven million inpatient stays yearly, includes 20% of admissions, and represents over 96% of the American population. Multivariate logistic regression, controlling for confounders, was conducted to explore associations between PA and delivery outcomes. According to the Tri-Council Policy Statement (2018), IRB-approval was not required, given data was anonymous and publicly available.
Main results and the role of chance: Women with PA were older(P=0.0001), more likely obese(10.8% vs. 3.6%), with higher rates of chronic hypertension(53.9% vs. 1.8%), thyroid disease(15.7% vs. 2.5%), pre-gestational diabetes(5.9% vs. 1%)(all P=0.0001), and were more commonly African American and not Hispanic(P=0.04) than the controls. There was no statistical difference between the two groups in the other demographic features including; income distribution(P=0.45), hospital type (P=0.63), rates of smoking(P=0.99), illicit drug use(P=0.73) or use of assisted reproductive technology(P=0.94). After adjustment for significant confounders women with PA were more likely to experience gestational hypertension(aOR 3.6 95%CI:1.6-8.1, P=0.001) and eclampsia(aOR 19.0, 95%CI:2.6-138.2, P= 0.004). Moreover, women with PA were more likely to deliver by operative vaginal delivery(aOR 9.7 95%CI:6.3-15.1, P=0.0001). However, there was no increased risk for preeclampsia in women with PA(aOR 1.47 95%CI:0.78-2.76, P=0.23). This finding was consistent even when not controlling for confounding effects including pre-gestational hypertention(OR 0.81 95%CI:0.26-2.56, P=0.72. There were no differences in the number of women with PPROM(P=0.81), preterm delivery(P=0.88), placental abruptio(P=0.7), maternal death(P=0.99), hysterectomy(P= 0.99), cesarean section(P=0.76), chorioamnionitis(P=0.99), postpartum hemorrhage (P=0.53), maternal infection(P=0.99), pulmonary embolism(P=0.99) or disseminated intravascular coagulation(P=0.99) between the two groups. Furthermore, there was no difference in other neonatal outcomes including: small for gestational age(P= 0.18), fetal demise(P=0.85) or congenital anomalies(P=0.15).
Limitations, reasons for caution: This is a retrospective analysis utilizing an administrative database that relies on data coding accuracy and consistency.
Wider implications of the findings: Women with PA were more likely to experience adverse pregnancy outcomes, including gestational hypertension, eclampsia, and operative vaginal deliveries. Neonatal complications were not increased in PA. Surprisingly, there was no increased risk for preeclampsia in women with PA, which needs to be further studied.
Trial registration number: NA

P-643 Is Euploid blastocyst number higher in luteal versus follicular phase? A case-control study of IVF outcomes of follicular versus luteal phase ovarian stimulation

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Study question: Is there a difference between IVF outcomes in patients undergoing follicular versus luteal phase ovarian stimulation in different menstrual cycles?
Summary answer: Number of euploid blastocyst were higher in luteal phase ovarian stimulation IVF cycles. All other outcomes were similar between follicular and luteal phase IVF cycles.
What is known already: It has been published that human beings can have two or three follicular recruitment waves as observed in animals studies a long time ago. From these findings, several recent studies showed that two egg retrievals at the same menstrual cycle, named as Duo Stim, optimize time and IVF outcomes in women with low ovarian reserve due to more eggs retrieved in a shorter period with consequently higher probability of having good embryos to transfer. However, there is no knowledge about diferences concerning IVF outcomes between folicular and luteal ovarian stimulation, performed at the same women in different menstrual cycles.

Study design, size, duration: Retrospective, case-control study in a single IVF center. One-hundred-two patients who had two IVF treatments – the first cycle initiating ovarian stimulation at follicular phase (FPS) and the second cycle initiating after a spontaneous ovulation at luteal phase (LPS) – in different menstrual cycles (until 6 months apart) between 2014 and 2020, were included. Statistical analysis was performed with Mann-Whitney test and was considered significant when p≤0.05. Data is represented as mean±SD.
Participants/materials, setting, methods: Patients underwent two IVF treatments in different menstrual cycles; the FPS IVF treatment was initiating at D2/D3 of menstrual cycle and the LPS treatment started three or four days after spontaneous ovulation, if at least 4 antral follicles were detected. Both IVF treatments were performed with and antagonist protocol and freeze all strategy. The majority of patients presents low ovarian reserve/Ovarian age as primary infertility factor (84.3%).
Main results and the role of chance: Patient's mean age was 39.30±3.15 years, BMI (22.66±3.16) and AMH levels (0.85±0.85 ng/mL). Comparison of hormonal levels at the beginning of ovarian stimulation showed differences for FPS vs LPS, as expected: E2 (39.69±31,10 pg/mL vs 177.33±214.26 pg/mL,p< 0.0001) and P4 (0.76±2.47ng/mL vs 3,00±5.00 ng/mL,p< 0.0001). However, E2 and P4 at the day of oocyte maturation trigger were not different between FPS and LPS (1355.24±895.73 pg/mL vs 1133.14±973.01 ng/mL,p=0.0883 and 1.12±1.49 ng/mL vs 2.94±6.51,p=0.0972 respectively). There was no difference for total dose of gonadotrofins (FPS 2786.43±1102.39.01UI vs LPS 2824.12±1188.87UI, p=0,8578), FSH (FPS 9.50±4.98 vs LPS 11.90±12.99,p=0.7502) and AFC (FPS 7.13±4.25 vs LPS 6.42±4.65,p=0,0944). From 102 patients that started ovarian stimulation, 78 had 1 or more oocyte collect in FPS group and 75 in LPS group: OPU (FPS 4.78±4.93 vs LPS 4.65±5.54,p=0.7889), number of MII (FPS 3.21±3.52 vs LPS 3.40±4.53,p=0.7889). From those, 52 patients performed ICSI in both cycles; fertilization rate 64.9%±28.6% for FPS vs 62.1%±32.4% for LPS,p=0.7899) and blastocyst formation 2.15±2.15 for FPS vs 2.54±2.35,p=0.3496). Data from 25 patients who had embryo biopsy for PGT-A showed similar number of blastocyst biopsed (2.12±1.72 FPS vs 2.48±1.71 LPS,p=0.3101) and a statistically significant difference regarding number of euploid blastocyst (0,20±0,41 FPS vs 0,96±0,93 LPS,p=0,0008).
Limitations, reasons for caution: This is a retrospective study in a limited number of patients. Therefore, it is not possible to make a definitive conclusion that LPS proportionate higher number of euploid than FPS. More studies are necessary to investigate not only IVF outcomes but also the impact on pregnancy rates.
Wider implications of the findings: In our study, LPS protocol after spontaneous ovulation, presents similar IVF outcomes compared to routinely FPS protocol. Intriguingly, the number of euploid blastocyst was significant higher in LPS, which may be further investigated. In this way, LPS is another option of IVF treatment, and may optimize time and treatment results.
Trial registration number: Not Applicable

P-644 Effect of a reduced dose of long-acting gonadotropin releasing hormone (GnRH) agonist versus short-acting GnRH agonist on pregnancy outcome in patients undergoing ICSI/ET cycles

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Study question: Are pregnancy outcome different between reduced dose of long-acting GnRH agonist and short-acting GnRH agonist in patients undergoing ICSI/ET cycles**?**
Summary answer: Clinical outcomes were not significantly different between the two group**.**
What is known already: There is no consensus on which of two GnRH agonist protocol is the most effective form and it has been challenging which one improves the clinical pregnancy rate.
Study design, size, duration: This is randomized clinical trial that 394 patients, who were candidates for ICSI/ET, were included in this study between April 2019 and January 2020.
Participants/materials, setting, methods: Patients were randomly divided into two groups using blocks of 8; hereby every 8 subjects were randomized, four were allocated to the long-acting GnRH agonist, and four were allocated to the short-acting GnRH agonist randomly**.**
Main results and the role of chance: No significant difference was noted between the two groups in progesterone and estradiol levels at hCG administration day. Despite no significant difference between the two groups in ART outcomes, interestingly rate of ovarian hyperstimulation syndrome (OHSS) occurrence was significantly higher in the short-acting group (P=0.005).
Limitations, reasons for caution: We couldn't evaluate the long-term consequence of pregnancy including live birth rate and effect of GnRH analogue on fetus.
Wider implications of the findings: A lower rate of OHSS occurrence in the long-acting group, and similar ART outcomes in both groups.
Trial registration number: IRCT20190609043845N1

P-645 Abnormal thyroid hormone levels are associated with biochemical primary ovarian insufficiency and low ovarian reserve: a cross-sectional study

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Study question: To address whether there was a correlation between thyroid parameters and primary ovarian insufficiency.
Summary answer: Thyroid dysfunction was related with bPOI and LOR.
What is known already: In our daily clinical work, there seems to be a link between thyroid disease and POI. A recent study reported that subclinical hypothyroidism was associated with lower ovarian reserve during later reproductive age. Animal studies demonstrated that thyroid hormones played important roles in ovarian functions, and both maternal hypothyroidism and hyperthyroidism were related with reduced primordial, primary and secondary follicle number in rats. However, others reported that thyroid autoimmunity (TAI) and hypothyroidism were not associated with DOR. In all, accumulative animal and epidemiological studies indicated the connection between thyroid function and ovarian reserve, but the results were still inconsistent.
Study design, size, duration: This is a cross-sectional study with consecutive women performed ovarian reserve assessment and thyroid test in the Second Hospital of Zhejiang University School of Medicine from April 2016 to March 2019. A total of 2109 women were included, with 111 with bPOI and 1771 without bPOI. To exclude the influence of age the participants were categorized into low ovarian reserve (LOR) and non-LOR groups based on age-specific AMH, including 78 LOR and 2031 non-LOR.
Participants/materials, setting, methods: At the outpatient, a doctor carried out an interview, recorded age, body mass index (BMI), past history, and current treatment, and made a diagnosis. Serum AMH, FSH, TPOAb, TgAb, Tg, TT3, FT3, TT4, FT4, and TSH levels were measured with electrochemiluminescence method.
Main results and the role of chance: TT3, FT3, FT4 was significantly positively correlated with serum AMH level. Further logistic regression analysis found that abnormal TT3, FT3 and TT4 levels were related to increased risks of bPOI and LOR. Chi-square analysis also proved that the incidence of abnormal TT3, FT3 and TT4 increased significantly in women with bPOI or LOR. The incidence of bPOI and LOR increased significantly in women with 2 or 3 abnormal thyroid hormones. The above analyses demonstrate in multiple aspects that thyroid dysfunction is related with decreased ovarian reserve.
Limitations, reasons for caution: Since this is a retrospective cross-sectional study, we only got the correlation between factors, and we could not achieve causal relationship. Further prospective cohort or randomized controlled trial (RCT) studies are required to make the results more robust.
Wider implications of the findings: The present study demonstrated that thyroid dysfunction was related with bPOI and LOR. It might be thyroid hormones, not TSH or thyroid antibodies, played the major role in ovarian reserve impairment. The treatment of euthyroxine may improve the ovarian reserve function.
Trial registration number: not applicable

P-646 Pregnancy outcomes in women with panhypopituitarism - A population-based study.

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Study question: What are the consequences of panhypopituitarism on pregnancy outcomes?

Summary answer: After controlling for confounding effects, women with panhypopituitarism have a higher prevalence of adverse obstetrical (including post-partum hemorrhage, hysterectomy and maternal death) and neonatal outcomes.

What is known already: Panhypopituitarism is a condition of inadequate or absent anterior pituitary hormone production. Pregnancy in women with panhypopituitarism is uncommon and there is only limited data (mainly case reports) regarding pregnancy outcomes in these women. Given the scarcity of data we sought to assess the association between panhypopituitarism and obstetrical and neonatal outcomes.

Study design, size, duration: A retrospective population-based study utilizing data from the Healthcare Cost and Utilization Project—Nationwide Inpatient Sample (HCUP-NIS). A dataset of all deliveries between 2004 and 2014 inclusively, was created. Within this group, all deliveries to women who had a diagnosis of panhypopituitarism during pregnancy were identified as part of the study group (n=179), and the remaining deliveries comprised the reference group (n=9,096,609).

Participants/materials, setting, methods: The HCUP-NIS is the largest inpatient sample database in the USA, and it is comprised of hospitalizations throughout the country. It provides information relating to 20% of US admissions and represents over 96% of the American population. Multivariate logistic regression analysis, controlling for confounding effects, was conducted to explore associations between panhypopituitarism and delivery and neonatal outcomes. According to Tri-Council Policy statement (2018), IRB approval was not required, given data was anonymous and publicly available.

Main results and the role of chance: Women with a diagnosis of panhypopituitarism were more likely to be older, to have a diagnosis of chronic hypertension, to have a diagnosis of pre-gestational diabetes mellitus and to be carrying twins or a higher order pregnancy (all p<0.0001), than the controls. A significantly higher risk of post-partum hemorrhage (adjusted odds ratio-aOR:3.52; 95%CI:2.18–5.69,p<0.0001), maternal infection (aOR:3.97; 95%CI:2.30–6.85,p<0.0001), pulmonary embolism (aOR:14.90; 95%CI:2.06–107.82,p<0.007), disseminated intravascular coagulation (aOR:20.29; 95%CI:10.60–38.85,p< 0.0001), maternal death (aOR:31.90; 95%CI:3.33–234.85,p=0.001) and congenital anomalies (aOR:4.55; 95CI:1.86–11.16,p=0.001), were found among the panhypopituitarism patients. Surprisingly, there was a lower incidence of caesarean delivery (aOR:0.69; 95%CI:0.50–0.96,p=0.026) in the panhypopituitarism patients than the controls. No significant difference was found in the rate of pregnancy induced hypertension (95%CI:0.78-1.97), gestational hypertension (95%CI:0.14-1.41), preeclampsia (95%CI:0.96-2.99), gestational diabetes (95%CI:0.30-1.01), preterm delivery (95%CI:0.74-1.91), preterm premature rupture of membranes (95%CI:0.17-2.82), operative vaginal delivery (95%CI:0.23-1.19), small for gestational age neonates (95%CI:0.27-2.02) or intra-uterine fetal demise (95%CI:0.13-6.71).

Limitations, reasons for caution: The limitations of our study are its retrospective nature and the fact that it relies on an administrative database. The severity of specific hormonal deficiencies and the presence and magnitude of posterior pituitary hormone deficiencies could not be assessed, nor could compliance with hormone replacement.

Wider implications of the findings: Until now, no control studies of outcomes with panhypopituitaryism in pregnancy are available in the medical literature. Physicians should be aware of and try to prevent the above possible maternal and fetal complications related to this endocrinopathy. Future studies should evaluate the role of medication compliance with pregnancy outcomes.

Trial registration number: not applicable

P-647 How do migraine attacks change during puberty?

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Study question: How do the stage of puberty and the menstrual cycle influence characteristics of migraine?

Summary answer: During puberty, the frequency of migraine attacks increases, especially during the follicular phase. The pattern of migraine changes to a typical adult pattern of migraine.

What is known already: Up to puberty, headaches are as common in girls as in boys. After the onset of puberty migraine is more prevalent in adolescent girls suggesting an association with sex hormones. Attacks of menstrual migraine are characterized by a longer duration, tend to be more severe, and are less responsive to acute medication compared to migraine attacks which are independent from the menstrual cycle phase.

Study design, size, duration: For this prospective cohort study 47 girls were recruited from two Departments of Pediatrics and Adolescent Medicine between 01/2016 and 12/2018.

Participants/materials, setting, methods: Girls between 7 and 18 years old, diagnosed with migraine without aura according to the "International Classification of Headache Disorders II" diagnostic criteria, took part. Three groups (pre-, peri-, and postpubertal) were formed according to the Tanner stage and the onset of a regular menstruation. Girls kept a daily headache and menstrual cycle diary over 8 weeks. Ovulatory cycles were analyzed by weekly progesterone saliva tests.

Main results and the role of chance: Three groups according to Tanner stage and onset of regular menstruation were compared: pre- (n=16), peri- (n=19) and post-pubertal (n=12) girls. A significant difference in migraine frequency was found between pre- and post- pubertal girls (p=0.005). Headache characteristics did not differ significantly between the three groups. Interestingly, a higher frequency of attacks in follicular phase occurred compared to luteal phase (p=0.030).

Limitations, reasons for caution: Repeated blood sampling would have been a more reliable technique compared to saliva assays. The sample size is small.

Wider implications of the findings: During puberty, the number of migraine attacks but not the specific headache characteristics changes in adolescent girls which should be taken into consideration regarding the management of these patients.

Trial registration number: AN2013-0027

P-648 In-vitro Evaluation of SCF and StxBP1 in Polycystic Ovary Syndrome (PCOS)

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Study question: Is the interaction between intrafollicular cells in PCOS, impaired by the change of vesicular fusion and/or exocytosis in granulosa cells (GCs)?

Summary answer: StxBP1 expression leves impared in GCs of PCOS.

What is known already: PCOS characterised as follicular arrest on antral follicles, cystic follicle formation, and follicular development failure. GCs secretes wide variety of factors via exocytosis, and plays critical role during

folliculogenesis. Secretory vesicles are transported to cellular membrane. This process requires local concentrations of SNAREs consisting of tSNARE, vSNARE, and other vesicle fusion associated proteins. SNARE proteins are involved in vesicle fusion, exocytosis, and intracellular trafficking. GCs secretes KITL which provides follicular activation and growth. Syntaxins are one of the members of SNARE complex. StxBP1 is a protein which has a crucial role in secretory vesicle fusion that provides fusion of syntaxins.

Study design, size, duration: Granulosa cells (GCs) were collected for primary cell culture, since 2019 from both PCOS (n=10) and healthy (male factor infertility) (n=10) women undergoing ART. Each GCs from participant divided into two groups as in-vitro stimulated group and in-vitro nonstimulated group.

Participants/materials, setting, methods: GCs have been isolated from follicular fluid taken from patients during oocyte pick-up at Hacettepe University In-Vitro Fertilization Unit. nGCs were cultured at most secod subcultures after the isolation. The stimulated groups of both PCOS and control groups were stimulated by hCG(10IU/ml) ve FSH(0.5IU/ml) for 24 hours. Vesicle fusion proteins (Stx6, StxBP1, and SNAP25), KITL, and FSHr expressions were analyzed on granulosa cells from each group via immunofluorescent (IF) labeling and cyto-ELISA.

Main results and the role of chance: FSHr were compared in both control and PCOS before and after stimulation. There was no difference between FSHr expression levels in both groups. Indirect IF is widely considered for SNAP25, Stx6, StxBP1 proteins in all groups of GCs screening with/without stimulation. Expression of SNAP25, StxBP1 mainly scattered through all cytoplasmic area,s and membranous localization was observed. Stx6 expressions were particularly distinguished at perinuclear area of cytoplasm. However, stimulated cells of control appeared more peripherally Stx6 expression. This pattern caused by stimulation wasn't observed in PCOS. Expressions of SNAP25, Stx6, StxBP1 were observed with less expression in PCOS. Also, the response to stimulation was lower than the control group. The differences in Stx6, SNAP25, StxBP1 and KITL levels before and after stimulation was evaluated for both control and PCOS in Cyto-ELISA. However, both SNAP25 and Stx6 expressions in GCs of both groups were similar in response to stimulation. The expression levels of StxBP1 in response to stimulation were significantly lesser than control at PCOS. KITL expressions were lower in the PCOS as expected furthermore similar to StxBP1 in response to stimulation. According to our findings, the highest response to stimulation in GCs occurred for StxBP1 and KITL in the control.

Limitations, reasons for caution: Since human cells were used in the study and the cells of each patient do not exhibit the same characteristics, the lowest number of patient samples identified in the statistical power analysis were included in the study.

Wider implications of the findings: Our view to the disruption in the secretion of signal molecules in terms of vesicle dynamics will offer a new perspective in female infertility or cross-talks in folliclar cells of the ovary.

Trial registration number: TSA-2019-18196

P-649 Should women receive luteal support following natural cycle frozen embryo transfer? A systematic review and meta-analysis

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Study question: Should women receive luteal phase support (LPS) following natural cycle frozen embryo transfer (NC-FET)?

Summary answer: Progesterone LPS following NC-FET increases the live birth rate. There is no evidence to support the administration of hCG for LPS in these cases.

What is known already: Whether or not women should receive LPS following NC-FET is highly controversial. Previous studies have shown conflicting results.

Study design, size, duration: We conducted a systematic search of the literature published in Medline/PubMed, Embase and the Cochrane Library, from January 2000 until December 2020. We included all original English, peer-reviewed articles, irrespective of study-design. The search strategy included keywords related to natural cycle frozen embryo transfer and luteal phase support. Studies reporting the results of artificial or stimulated FET cycles were excluded.

Participants/materials, setting, methods: Our systematic search generated 395 records. After screening, eight studies were included in the review and seven studies were included in the meta-analysis. Two studies (n=858) used hCG, and 6 studies (n=1507) used progesterone for luteal support. Four studies were randomized controlled trials (RCTs), whereas the other four were historic cohort studies.

Main results and the role of chance: In a meta-analysis using random effects model, hCG administration for LPS did not increase the clinical pregnancy rate (two studies, OR 0.85, 95% CI 0.64-1.14). On the other hand, progesterone LPS was associated with a higher clinical pregnancy rate (five studies, OR 1.48, 95% CI 1.14-1.94), and a higher live birth rate (three studies, OR 1.67, 95% CI 1.19-2.36).

Limitations, reasons for caution: There was large heterogeneity in progesterone dose and route of administration, as well as the methods used for ovulation detection and triggering. Moreover, only four studies were randomized. Finally, both studies examining the use of hCG for LPS were performed by the same group of researchers in a single center.

Wider implications of the findings: The available evidence indicates that progesterone administration for LPS is beneficial following natural cycle frozen embryo transfer. There is no evidence to support the administration of hCG for LPS in these cases. Additional Large RCTs are necessary in order to improve the quality of evidence and validate our findings.

Trial registration number: PROSPERO ID: CRD42020199045

P-650 Body weight is the main determinant of systemic follicle stimulating hormone (FSH) concentrations during controlled ovarian stimulation with follitropin delta

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Study question: Which individual subject characteristics affect systemic FSH concentrations in women undergoing controlled ovarian stimulation with follitropin delta?

Summary answer: Body weight is the main determinant of systemic FSH concentrations. Renal function, Asian race, country/region, hepatic function and age have at most a small influence.

What is known already: After administration of FSH, systemic FSH concentrations are inversely related to body weight. It has been observed that the impact of body weight on ovarian response is clinically relevant at low doses but not at high doses. In patients with anti-Müllerian hormone (AMH) ≥15 pmol/L, follitropin delta is dosed according to each patient's body weight, which influences systemic FSH concentrations, and her AMH level which predicts ovarian response.

Study design, size, duration: Serum FSH concentrations were assessed in five randomised, controlled, assessor-blinded, multicentre trials of follitropin delta in women undergoing an assisted reproductive technology programme. The trials were conducted in Europe, America and Asia. In all, 1.665 women treated with follitropin delta contributed to the evaluation with 4052 serum FSH concentrations, measured at steady state by an immunoassay.

Participants/materials, setting, methods: FSH concentrations were described with a pre-specified one-compartment population pharmacokinetic model. The key model parameters were the apparent total clearance (CL/F) of follitropin delta, the interindividual variability herein and the effects of baseline values of body weight, age, race, country/region, renal and hepatic function on CL/F. Renal function was assessed using the estimated glomerular filtration rate (eGFR) and hepatic function by alanine transaminase (ALT) and bilirubin levels.

Main results and the role of chance: The area under the FSH concentration-time curve during a dosing interval (AUC) was derived from dose and CL/F. Body weight was the covariate with the most pronounced effect on AUC, both in terms of the effect magnitude and statistical significance. AUC was 1.51-fold higher (90% confidence limits: 1.48; 1.54) in women with the lowest observed body weight of 40 kg compared to women with a typical body weight of 58 kg. The effect of renal function on AUC was small and in the same order of magnitude as the bioequivalence limits (0.8; 1.25). AUC was 1.28-fold higher (90% confidence limits: 1.23; 1.33) in women with the lowest observed eGFR value of 44 mL/min/1.73m2, compared to women with a typical eGFR value of 98 mL/min/1.73m2. The effects of Asian race and country/region (Japan, China, Other Asian) were confounded with each other and well within the

bioequivalence limits when evaluated independently. The effects of age and the hepatic function markers ALT and bilirubin were well within the bioequivalence limits.
Limitations, reasons for caution: The women participating in the trials were generally healthy and the results cannot be transferred to women with renal or hepatic disease. A limited number of Black women contributed to the present analysis but the trend was similar. Data is forthcoming from ongoing trials including larger numbers of Black women.
Wider implications of the findings: The findings support dosing follitropin delta by body weight and without adjustment for renal function, hepatic function, race, age or country/region.
Trial registration number: NCT01426386, NCT02309671, NCT01956110, NCT03228680 and NCT03296527

P-651 Strict embryo-endometrial synchrony does not contribute to the successful pregnancy during vitrified-warmed embryo transfer with hormone replacement cycles

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Study question: Does strict embryo-endometrium synchronization relate to pregnancy during vitrified-warmed embryo transfer (ET) with hormone replacement (HRT) cycles?
Summary answer: A 12-hour delay in the embryo-endometrial synchrony was acceptable, and this delay was not an independent predictor of pregnancy during vitrified-warmed ET with HRT cycles.
What is known already: Embryo-endometrium synchrony is considered to be necessary for successful pregnancy in both fresh and frozen-thawed cycles. Until now, the date of ET has been determined by the synchronization of the embryo developmental stage and the endometrium on a daily basis. To date, with the advent of the time-lapse incubator, it is possible to observe the embryo development from fertilization over time and to calculate the exact time from fertilization of the transferred embryo. However, there are very few studies on the extent to which increases the accuracy of synchronization between embryo and endometrium contributes to a successful pregnancy.
Study design, size, duration: This retrospective cohort study included 319 consecutive cycles during vitrified-warmed ET with HRT between August 2016 and August 2018. This study was conducted in an academically affiliated private practice.
Participants/materials, setting, methods: We analyzed 319 vitrified-warmed single-blastocyst transfer cycles. All frozen expanded blastocysts were inseminated by intracytoplasmic sperm injection (ICSI) and cultured in a time-lapse incubator. We calculated time for the in vitro culture of the embryo after ICSI (t1) and time for progesterone-priming (t2) up to ET. The difference between t1 and t2 (delta-t) was used as an indicator of embryo-endometrium synchrony. We examined the relationship between delta-t and treatment outcomes using multivariate logistic analysis.
Main results and the role of chance: The mean patient's age at oocyte retrieval was 35.7 (SD 4.3). The number of pregnant cycles was 157 in all treatment cycles (pregnancy rate, 49.2%). The mean value of delta-t was 9.9 h (SD 2.6) in all cycles. There was no significant difference of delta-t in pregnant (mean, SD: 10.0 h, 2.8 h) and non-pregnant cycles (mean, SD: 10.0 h, 2.3 h). Treatment cycles were classified according to the quartile of delta-t, and we examined the percentages of pregnant cycles in each group. There were no significant differences in pregnancy rates among the groups (p=0.75). On multivariate logistic analysis, patient's age (adjusted odds ratio [aOR]: 0.94, 95% confidence interval [CI]: 0.89–0.99), previous treatment cycles (aOR: 0.74, 95% CI: 0.56-0.99), endometrial thickness at ET (aOR: 1.19, 1.04–1.36), and good quality blastocysts (>3BB according to Gardner's classification) at vitrification (aOR: 2.49, 95% CI: 1.23–5.05) were independent predictive factors for pregnancy. On the other hand, delta-t did not contribute to pregnancy (aOR: 1.00, 95% CI: 0.99–1.00).
Limitations, reasons for caution: We did not examine the effects of embryo-endometrium synchrony during vitrified-warmed ET in a natural cycle. Therefore, careful interpretation of the significance of embryo-endometrium synchrony during the vitrified-warmed ET should be taken.
Wider implications of the findings: We showed the embryo-endometrium synchrony did not contribute to the pregnancy during vitrified-warmed ET with HRT cycles. These results cast doubt on the existence of an optimal implantation window by changing the timing of ET with the results of gene expression testing of the endometrium.
Trial registration number: Not Applicable

P-652 Higher live birth rates with controlled ovarian stimulation vs. natural cycles in donor sperm IUI

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Study question: Are pregnancy rates after intra uterine insemination-donor sperm (IUI-D) in good prognosis patients with controlled ovarian stimulation (COS) different from those in natural cycles?
Summary answer: In good prognosis patients, IUI-D cycles with COS provided higher pregnancy outcomes compared to IUI-D in natural cycles.
What is known already: There is no consensus about the systematic use of COS for IUI-D in good prognosis patients, considering efficacy, safety, and efficiency. The objective of this study is to compare the clinical pregnancy rate in good prognosis patients undergoing an IUI-D cycle with COS versus natural cycle (NC).
Study design, size, duration: Retrospective cohort study of 5,369 first IUI-D performed between January 2012 and September 2019 in one fertility center. IUI-D with COS (n=4,417) versus natural cycles (n=952) were compared. Differences in pregnancy outcomes between study groups were evaluated using a Pearson's Chi2 test. A $p<0.05$ was considered statistically significant.
Participants/materials, setting, methods: Good prognosis patients were defined as women aged ≤38 years old, with a BMI ≤35 Kg/m2, and having regular menses. The indications for IUI-D were an absence of male partner or a sever partner male factor. COS consisted in a standard protocol of r-FSH or hMG-HP, in a dose between 25 IU to 75 IU, depending on the patient's age and the acceptance of multiple pregnancy, to obtain between 1 to 2 follicles at ovulation.
Main results and the role of chance: Average age was slightly higher in COS patients (33.0±3.8 versus 31.6±4.1 years old in NC), as was BMI (23.7±3.6 in COS vs 23.08±4.1 in NC). Further, in the last follicular control, estradiol was higher (321±180 vs 244±108 pg/ml), LH was lower in (14 vs 28 UI/L), and the number of follicles > 16mm was higher (1.06±0.5 vs 0.96±0.4) in COS vs NC, respectively. Progesterone levels did not differ between groups. Stimulated cycles provided significantly better results for all pregnancy outcomes ($p<0.001$): biochemical pregnancy rate was 27.8% in COS versus 23.0% in NC; clinical pregnancy rate was 20.5% versus 14.8%; ongoing pregnancy rate was 18.5% versus 13.3%; and live birth rate was 16.8% versus 12.3%. While the analysis was not adjusted for potential confounding factors, baseline characteristics between groups were very similar, so we could expect that the improved reproductive results were due to COS.
Limitations, reasons for caution: The main limitation of the study is its retrospective nature and the collection of data from one clinic. Differences found between study groups should be confirmed in a prospective controlled trial.
Wider implications of the findings: In good prognosis patients undergoing their first IUI-D, controlled ovarian stimulation provides better reproductive outcomes; further analysis of cumulative pregnancy rate after 3 cycles would provide information for recommendations on the complete treatment cycle.
Trial registration number: non applicable

P-653 The levels and ratios of adipokines in the follicular fluid as promising prognostic factors for in vitro fertilization outcomes

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Study question: Can the adipokines levels and ratios in the follicular fluid (FF) be used to predict in vitro fertilization (IVF) outcomes?
Summary answer: The leptin level and ratios leptin/ghrelin and leptin/adiponectin in FF are reliable prognostic factors for IVF outcomes in women with normal body mass index (BMI).
What is known already: The adipose tissue serves not a simple fat storage, rather an important endocrine organ, producing adipokines, such as leptin, adiponectin, ghrelin and others. Adipokines have been shown to regulate the cardiovascular system, food intake, metabolism, inflammation, metastatic spread of tumors,and also reproduction, affecting the activity of the hypothalamo-pituitary-gonadal axis. The plasma and FF adipokines have been used as prognostic factors for IVF outcomes, but the obtained results are controversial. The most promising in this case may be the distribution of patients into groups in accordance with their BMI and a separate study of adipokine ratios in them.
Study design, size, duration: Women (n=53), who undergo IVF, were divided on two groups, according to their BMI: normal BMI (18.5-24.9 kg/m2, n=25) and increased BMI (>25.0 kg/m2, n=28). Depending on IVF outcomes after the antagonist protocol, the groups formed were subsequently subdivided into two groups each: non-pregnant with normal BMI (nPN, n=16), pregnant with normal BMI (PN, n=9), non-pregnant with increased BMI (nPI, n=21), pregnant with increased BMI (PI, n=7). Participants/materials, setting, methods: Inclusion criteria for participants were: age 18-45 years and infertility due to male/tubal factor. Exclusion criteria were: polycystic ovarian syndrome (PCOS), diabetes mellitus, or plasma level of anti-Müllerian hormone <1.0 ng/mL. The FF from the first punctated follicle was collected and tested for leptin, adiponectin and ghrelin levels using ELISA kits. If gestational sac was seen in uterine cavity using ultrasound on day 21-25 after embryo transfer, pregnancy was diagnosed.
Main results and the role of chance: Women with increased BMI had, as a rule, higher FF levels of leptin and the leptin/ghrelin and leptin/adiponectin ratios, compared with women with normal BMI. Furthermore, leptin level was higher in PN as compared to nPN (21.61±2.55 vs 10.54±1.16, p<0.05), but did not differ in the PI and nPI groups. The same pattern was obtained for the leptin/ghrelin and leptin/adiponectin ratios. The ghrelin level showed an inverse pattern. It was higher in nPN as compared to PN (3.80±0.78 vs 1.39±0.19, p<0.05), but did not differ in women with increased BMI. The obtained data demonstrate that the association between the adipokine levels in FF and the IVF outcomes is better in women with normal BMI than in women with increased BMI. Adipokines, which differed among the study groups, were evaluated as prognostic factors in women with normal BMI.The values were calculated at which pregnancy became unlikely: leptin level <15 ng/mL, leptin/ghrelin ratio <9, and leptin/adiponectin ratio <5. For each indicators, the sensitivity and specificity were >88%. The predictive power of these indicators in the clinic using the odds ratio (95% confidence interval) was: leptin level 7.11 (1.23-40.99, p<0.05), leptin/ghrelin 29.53 (1.53-570.83, p<0.05), and leptin/adiponectin 7.11 (1.23-40.99, p<0.05).
Limitations, reasons for caution: The main limitation of the study is a relative small number of patients, although the differences between the investigated groups were significant. Furthermore, women with low ovarian reserve, age > 40 years, endometriosis or PCOS were not included in the study.
Wider implications of the findings: The obtained results indicate the good prospects for using such indicators as the adipokines levels and their ratio in FF to predict IVF outcomes in women with low ovarian reserve, endometriosis and PCOS, but with normal BMI.
Trial registration number: Not applicable

P-654 What is the best luteal phase support in frozen-thawed embryo transfer cycle?

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Study question: What is the best progesterone administration for luteal phase support (LPS) in frozen-thawed embryo transfer cycle?
Summary answer: Different modes of hormonal luteal phase support do not affect clinical pregnancy rate (CPR) or live birth rate (LBR) in frozen-thawed embryo transfer (FET) cycles.
What is known already: FET has increased substantially over the last years. To support implantation, endometrial and embryo maturities must be synchronized; therefore, endometrial and follicular maturation are monitored either within the artificial cycle. Estrogen and progesterone are sequentially administered. The optimal endometrial preparation strategy remains unclear; this study aims to compare the reproductive and pregnancy outcomes between five different regimens of hormonal LPS for FET treatment.
Study design, size, duration: Single centre retrospective cohort study conducted between 2013 and 2019. Included were women (N=402) aged 18-45 years undergoing FET. After an optimal endometrial preparation and endometrial thickness, the LPS was started. Thereafter, five different progesterone applications were compared: (1) oral dydrogesterone (10mg tid), (2) vaginal progesterone gel (90mg/d), (3) dydrogesterone (10mg tid) plus vaginal progesterone gel (90mg/d), (4) vaginal progesterone capsules (200mg tid), or (5) subcutaneous injection of 25mg daily.
Participants/materials, setting, methods: An ultrasound was performed 14 days of estrogene (>=4mg/d) preparation. If the endometrial thickness was ≥7 mm and there was no dominant follicle, luteal support commenced four days before FET. Fourteen days after transformation, a serum beta-hCG test was performed. If positive (> 50 IU/L), a transvaginal ultrasound was performed to confirm clinical pregnancy, defined as gestational sac with fetal heart movement. CPR was assessed and delivery reports for LBR were collected later.
Main results and the role of chance: In total, 402 patients on an artificial cycle were included (mean age, 35 years (y); range, 26-46 y; standard deviation, 4.1 y). No differences in endometrial thickness and cause of infertility were found between groups. Multivariate logistic regression analysis revealed that the odds ratios (ORs) with 95% confidence intervals (CIs) for the CPR was significantly higher in the dydrogesterone only group (OR, 3.25; 95% CI, 1.7-6.2; p < 0.001) and in the combined group (3) (OR, 7.55; 95% CI, 2.7-21.10; p < 0.001). Statistically significant differences in live birth rate could not be found between the five groups; they were 33%, 54%, 8.3%, 4%, 0% for groups 1, 2, 3, 4, and 5 respectively. Overall satisfaction and tolerability were significantly higher in oral dydrogesterone compared to the vaginal progesterone.
Limitations, reasons for caution: This is a retrospective single-center study. Also, potentially confounding variables like ethnicity, parity, BMI were not accounted for, possibly contributing to bias. Further prospective randomized studies are needed.
Wider implications of the findings: Oral dydrogesterone was found to be effective with equal CPR and LBR. Benefit is well-tolerated and accepted among patients; however we did not observe significant differences in the rates of live birth between the five regimens for used for LPS in women undergoing frozen-thawed embryo transfers.
Trial registration number: BASEC Switzerland 2020-01527

P-655 Modelling patient selection in fresh vs. frozen-thawed IVF cycles in poor prognosis patients

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Study question: What is in poor-prognosis patients the impact of patient selection on outcome comparisons between fresh and frozen-thawed in vitro fertilization (IVF) cycles?
Summary answer: In poor prognosis patients, all-freeze cycles offer no outcome benefits and may, indeed, adversely affect IVF outcomes.

What is known already: Some in primarily good prognosis patients performed studies have suggested improved IVF outcomes if in stimulated cycles all embryos are cryopreserved (all-freeze cycles) and, after a time interval, in a later thaw cycle transferred. All-freeze cycles, therefore, despite considerable criticism, have become increasingly popular.
Study design, size, duration: Retrospective controlled cohort study involving 4 different patient pairings of 1st IVF cycles at our center between 2017-2020.
Participants/materials, setting, methods: n an academically affiliated private fertility and research center, we compared: (i) 127 fresh vs. 193 frozen donor recipient cycles; (ii) 741 fresh autologous vs. 217 frozen autologous cycles; (iii) 143 favorably selected autologous vs. same 217 frozen autologous cycles; and (iv) 598 poorer prognosis autologous vs. same 217 frozen autologous cycles. Main outcome measures were standard IVF outcomes.
Main results and the role of chance: Even in poor prognosis patients, patient selection to significant degrees impacts how fresh and frozen-thawed IVF cycles compare. Though the concept of embryo freezing with delayed embryo transfer may to a marginal degree beneficially affect IVF outcomes in best-prognosis patients, in unselected patients it has no effect on outcomes, while in poor-prognosis patients it adversely affects IVF outcomes. Unexpectedly, we also discovered a substantial impact of recipient age in donor-egg cycles on IVF outcomes because of age-associated increases in miscarriage rates independent of embryo aneuploidy.
Limitations, reasons for caution: Like in all retrospective studies, unknown patient selection biases cannot be ruled out. Moreover, because of the highly atypical characteristics of our center's patient population, generalization of here reported outcomes to more typical IVF patient populations must be viewed with caution.
Wider implications of the findings: In poor-prognosis patient, all-freeze IVF cycles should be avoided and considered contraindicated since they reduce outcomes. Since only best-prognosis patients benefit, with intermediate-prognosis, the practice should also be avoided. Unexpectedly, this study for the first time also revealed in recipients increasing miscarriages with advancing age (obviously not chromosomal).
Trial registration number: NA

P-656 Examination of the clinical significance of the two-step ovulation induction method (DuoStim)

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Study question: Do culture results of eggs obtained by double stimulation (DuoStim), where eggs are collected twice in one cycle, differ from a conventional fertility drug method?
Summary answer: The culture results of eggs acquired via the DuoStim cycle versus those acquired via a widely used conventional fertility drug method did not differ significantly.
What is known already: For patients with reduced ovarian reserve, the random start method, in which ovarian stimulation can start at any time during the menstrual cycle, is being used. As the pituitary gland is suppressed by progesterone during the luteal phase, endogenous luteinizing hormone surges are less likely to occur and ovulation is more easily avoidable. Previous reports showed that ovarian stimulation during the follicular and luteal phases of the same menstrual cycle resulted in similar blastocyst formation rates with normal chromosome numbers, which seems to be time-consuming. The DuoStim method is considered useful in cases in which time is at a premium.
Study design, size, duration: Between June 2019–December 2020, 562 egg collection cycles were performed in women ≥36 years. Ovulation cycles were evaluated in the conventional ovulation induction cycle (Co) group and DuoStim cycle (DS) group (subclassified into D1 group [first egg collection in cycle] and D2 group [second egg collection]. Post-insemination culture results were evaluated.
Participants/materials, setting, methods: Participants were women ≥36 years. Infusion method was IVF, and blastocysts of Gardner classification 3BB or higher were designated as good blastocysts, and blastocysts of 3AA or higher were designated as the best blastocysts. Confirmation of the fetal sac was defined as clinical pregnancy for the single freeze-thaw blastocyst transplant cycle. Chi-square and *t*-tests were used for statistical analysis. P≤0.05 indicated statistical significance.
Main results and the role of chance: The average number of eggs acquired per cycle was 6.9 in the Co group and 3.5 in the DS group, and the egg maturation rate was 88.0% in the Co group and 95.7% in the DS group, which showed significant differences. The 2PN rate, blastocyst arrival rate, and Day 5 good blastocyst arrival rate in the obtained mature eggs were 66.5%, 66.5%, and 38.3% in the Co group and 70.9%, 70.5%, and 34.4% in the DS group and were not significantly different. Similarly, when a comparative study was conducted between the D1 group and D2 group, rates were 67.5%, 69.0%, and 31.0% in the D1 group and 74.4%, 71.9%, and 37.5% in the D2 group, with no significant difference noted. Rates of clinical pregnancy and post-transplantation miscarriage were 41.1% and 17.8% in the Co group and 16.6% and 0% in the DS group, respectively, with no significant difference, although rates in the Co group tended to be better.
Limitations, reasons for caution: The fertilization method was evaluated only by IVF. The transplantation method was freeze-thaw embryo transfer by hormone replacement cycle, and the target age was 36 years or older.
Wider implications of the findings: DuoStim, which increases the number of acquired eggs, is useful when eggs must be collected as soon as possible. Regarding the clinical pregnancy rate after transplantation, better results were obtained for eggs acquired by the conventional fertility method, but it was necessary to repeat the number of attempts.
Trial registration number: not applicable

P-657 Prostaglandin D2 is correlated with follicles development and a reliable marker of ovarian reserve of poor ovarian responder patients

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Study question: Is the prostaglandin D2 (PGD2) associated with growing follicles and ovarian reserve of poor ovarian responders?
Summary answer: PGD2 is correlated with ovarian stimulation activity and follicle growth. Especially, poor ovarian responders show a significant decrease in the level of follicular fluid.
What is known already: Prostaglandins (PGs) are involved in the female reproductive process, mainly ovulation, fertilization, and implantation.
Study design, size, duration: We investigated the PGD2 level in the follicular fluid of poor ovarian responders. The collection of human follicular fluid was approved by the Institutional Research and Ethical Committees of CHA University (approval number: 1044308-201611-BR-027-04) from January to December 2019. Follicular fluid was collected from patients with normal ovarian response and patients with POR.
Participants/materials, setting, methods: We studied whether prostaglandin has related to POR in the clinical key factor by measuring human follicular fluid. Follicular fluid was collected from patients with normal ovarian response and patients with POR. The concentration of PGD2 in follicular fluid was determined with ELISA kits following the manufacturer's protocol.
Main results and the role of chance: We analyzed the level of PGD2 in the follicular fluid of patients with normal ovarian response and patients with POR using an ELISA. The PGD2 concentration was significantly lower in the follicular fluid of patients with POR than in the follicular fluid of young and old patients with normal ovarian response.
Limitations, reasons for caution: This study has an identification of biomarker of the clinical samples as POR criteria patients. Therefore, further investigations aimed at specific recovery of low PGD2 metabolic activity in the CCs during control ovarian stimulation.
Wider implications of the findings: Until now there is no specific biomarker of POR. AMH is just an ovary reserve marker for an indication of ovary function. PGD2 is one of the metabolites in steroid metabolism in the ovary. Therefore, we can find some cure through further study for improved PGD2 production to POR patients.
Trial registration number: none

P-658 Lovastatin promotes the expression of LDL receptor and enhances E2 production in the cumulus cells

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Study question: Lovastatin enhanced E2 productive ratios in the cumulus cells through promoted expression of Low-density lipoprotein receptor (LDLR).
Summary answer: Lovastatin up-regulated gene expression of LDLR in the CCs. And the high expression of LDLR promoted E2 productive ratios from CCs.
What is known already: We already reported that the up-regulation of LDLR correlated with clinical pregnancy. Therefore, we found lovastatin as an up-regulator of LDLR expression of clinical pregnancy.
Study design, size, duration: This is an expended study of LDLR to enhance steroidogenesis regarding the effect of lovastatin in the CCs. The collection of human cumulus cells was approved by the Institutional Research and Ethical Committees of CHA University (approval number: 1044308-201611-BR-027-04) from January to December 2019. The CCs were collected from 12 patients with normal ovarian response after oocyte denudation for ICSI.
Participants/materials, setting, methods: We studied whether lovastatin has up-regulated LDLR expression in human CCs. Cumulus cells were collected from patients with young (~ 36) and old aged patients (37 ~). After culturing human CCs, they were treated lovastatin for one day. The concentration of E2 in culture medium was measured using Chemiluminescence immunoassay. The mRNA isolated from CCs was analyzed gene expression level through real time-PCR.
Main results and the role of chance: The concentration of E2 was significantly increased in the culture medium treated with lovastatin. The CCs treated with lovastatin increased the expression of LDLR and StAR which are components of the steroidogenesis pathway.
Limitations, reasons for caution: We have found that the role of lovastatin promotes the E2 production by increasing the ldlr gene of CCs. Therefore, further investigations aimed at lovastatin effect on human oocytes embryo whether enhanced quality of oocytes or not.
Wider implications of the findings: Previous data show that high activation of LDLR and StAR was associated with embryo quality and clinical pregnancy in infertile women. Our data suggest that lovastatin is stimulated LDLR expression to enhanced pregnancy ratios of IVF patients.
Trial registration number: none

P-659 Artificial intelligence (AI) as an assisting tool in generating patient-friendly corifollitropin alfa ovarian stimulation protocol during in vitro fertilization

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Study question: How machine learning assisted in generating patient-friendly corifollitropin alfa protocol in normal responders?
Summary answer: In retrospective experiments, our machine learning model integrated physiological measurements of patients and clinical experience to generate a patient-friendly corifollitropin alfa protocol.
What is known already: Long-acting corifollitropin alfa can simplify the regimen, minimizing injections during the whole cycle. The previous study has described the patient-friendly protocol using corifollitropin alfa without routine pituitary suppression in normal responder can result in non-compromised clinical outcomes. Some studies showed machine learning can help with making clinical decisions and have the ability to learn from physiological measurements. Those methods effectuate certain points throughout short-acting menotropin protocols, however, there are still no robust AI tools for long-acting corifollitropin alfa protocols.
Study design, size, duration: 1,309 cycles were collected at Stork Fertility Center from November 2016 to October 2019, and 1,221 cycles were available after data cleaning and applying exclusion criteria, which Anti-Mullerian Hormone (AMH) is lower than 2. The data from electronic medical records (EMRs) consisted of age, AMH, body weight, luteinizing hormone (LH), and estradiol (E2) concentrations measured on revisit. Evaluation is performed by one physician who has more than 20 years of experience in IVF. Participants/materials, setting, methods: The protocol generator consisted of 5 parts: doses of Elonva, trigger type, doses of recombinant follicle-stimulating hormone (rFSH), doses of recombinant luteinizing hormone (rLH), and day of oocyte retrieval. The protocol was predicted by age, AMH, and weight firstly, then fine-tuned by LH and E2 after the first revisit. We used the gradient boosting decision tree algorithm to learn the protocol. The dataset was randomly split into 80% for training and 20% for testing.
Main results and the role of chance: In classification, the model predicted the dose of Elonva achieved an accuracy of 0.913 and an AUC of 0.946, and trigger type got an accuracy of 0.901 and AUC of 0.852 only using features on stimulation day (SD) 1 and gained 0.012 and 0.056 in accuracy and AUC correspondingly after adding features on the first revisit day. In regression, the mean absolute error (MAE) of rFSH dose, rLH dose, and oocytes retrieved day was 156.30 IU, 232.75 IU, and 0.80 days respectively, and after refining, the MAE dropped to 92.37 IU, 100.07 IU, and 0.46 days. The error of predictions in rFSH and rLH was almost equal to half increments of rFSH (150 IU) and one increment rLH (75 IU). This indicated that our model could provide a better prediction of these clinical decisions with one revisit only.
Limitations, reasons for caution: The present study was a single-center retrospective, and only analyzed the data from normal responders, whose AMH was equal or greater than 2. Though, the recommendations of our system act as references, the physician will make the final decision.
Wider implications of the findings: Our result showed the potential of machine learning in generating protocols is promising. Recommendations generated by our model can provide the junior clinical teams to optimize the clinical plans and learn from the experience of experts. We look forward to applying our machine learning model to different protocols.
Trial registration number: not applicable

P-660 Impact of elevated late-follicular phase serum estrogen and progesterone levels on blastocyst utilization and cumulative live birth rates in freeze-all cycles

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Study question: Does elevated late-follicular phase estrogen and progesterone levels have an impact on blastocyst utilization and/or cumulative live birth rates in freeze-all cycles?
Summary answer: High estrogen or progesterone on the day of ovulation trigger is associated with poor blastocyst utilization but comparable cumulative live birth rates in freeze-all cycles.
What is known already: Several studies suggest impaired clinical outcome in cycles with high estrogen (>3500 pg/ml) or progesterone (>1.5 ng/ml) levels. However, these data were derived from cycles where top-quality embryo(s) were transferred in the fresh cycle and surplus embryos were frozen. These findings might be confounded by alterations in endometrial receptivity. Freeze-all cycles might provide a better model to assess the impact of high late-follicular estrogen or progesterone levels on laboratory and clinical outcome.
Study design, size, duration: We performed a retrospective cohort study of all IVF cycles (n=712) between 2016 and 2018 where the entire cohort of embryos was cryopreserved at the blastocyst stage. After excluding cases with <4 oocytes or preimplantation genetic test, the study group comprised 459 women who had 699 frozen-thawed embryo transfer cycles.
Participants/materials, setting, methods: Women were classified into four groups by the indication for freeze-all strategy as elevated progesterone (high P, n=61), high estrogen (high E, n=224), elective freezing (elective, n=114) and tubal-endometrial pathologies (TEP, n=60). The primary outcome was the cumulative live birth rate in subsequent thaw-transfer cycles and the secondary outcome was the blastocyst utilization rate. Groups were compared using ANOVA and Cox regression analyses to adjust for confounding variables.

Main results and the role of chance: The mean age of the study group was 32.8 ± 5.3 years, total number of oocytes and cryopreserved blastocysts were 15.0±7.6 and 4.2±3.0, respectively. The high-E group was younger (31.5 ± 5.2 years) and had higher peak E2 levels (4078.9 ± 588.4 pg/ml), number of oocytes (19.7 ± 7.0), cryopreserved embryos (5.3 ± 3.3) and transfer cycles (2.3 ± 1.4) than the other groups. Blastocyst utilization rate was significantly lower (40.4%) compared to elective freezing (53.6%) and TEP groups (55.7%) (both p=0.001). The high-P group had higher peak progesterone levels (2.1 ± 0.5 ng/ml, p=0.001), number of oocytes (14.0 ± 5.2) and frozen embryos (4.1 ± 3.5) compared to elective and TEP groups (both p=0.04). Blastocyst utilization rate was lower (45.7%) than elective freezing and TEP groups but the difference lacked statistical significance (p=0.33 and p=0.21, respectively). Cumulative live birth rates were 42.6% in high-P, 59.8% in high-E, 44.7% in elective freezing and 46.7% in TEP groups. Significant predictors of cumulative live birth were female age (aHR: 0.97, 95%CI:0.95-0.99, p=0.02) and number of frozen blastocysts (aHR:1.05, 95%CI:1.01-1.10), p=0.02). When adjusted for these confounders, the cumulative live birth rate was not associated with high-E (aHR: 0.86, 95%CI:0.56-1.31) or high-P (aHR: 0.76,95%CI:0.44-1.32).

Limitations, reasons for caution: This was a retrospective study with small sample size performed at a single fertility center, which may limit the generalizability of our findings.

Wider implications of the findings: While lower blastocyst utilization rates are observed in women high late-follicular estradiol or progesterone levels, cumulative live birth rates in subsequent thaw-transfer cycles were not impaired. However, unfavorable outcome parameters observed in women with elevated progesterone deserve further research.

Trial registration number: Not applicable

P-661 Comparative assessment of the structural features of human follicle-stimulating hormone in products from multiple markets

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Study question: The aim of the study is to explore the structural differences occurring in recombinant human follicle-stimulating hormone alfa (r-hFSH-α), originator and its biosimilars, from various countries.

Summary answer: When compared with r-hFSH- originator (Gonal-*f*), its biosimilars presented structural differences, namely Primapur showed a significant different glycosylation profile.

What is known already: FSH is part of cystine knot growth factor superfamily and plays a central role in reproduction, as FSH stimulates follicular development and estrogen synthesis. R-hFSH-α is commonly used in assisted reproductive technologies to achieve multifollicular development. At the present r-hFSH-α biosimilars are available in Europe and other regions. R-hFSH- is a complex glycoprotein, that possesses several structural features critical for its efficacy and safety1-2. Glycosylation profile is one of the most impactful attributes of the molecule defining a moiety of FSH isoforms with impact on its biological net effect3. Efficacy and safety of r-hFSH-α are strictly correlated with glycoforms' composition3-8.

Study design, size, duration: At least two different batches of each r-hFSH-α originator and its biosimilars have been included in the study.

Participants/materials, setting, methods: The structural features of products from six different marketed r-hFSHα (Gonal-*f*, Primapur, Folisurge Intas, Corneumon, Jin Sai Heng, Follitrope LG) have been investigated with a variety of analytical techniques in order to evaluate the presence of molecular differences, which could have a severe impact on the efficacy and safety of the product. The attributes which have been investigated in-depth include primary, secondary and tertiary structure as well as post-translational modifications (PTMs), including glycosylation and contaminants.

Main results and the role of chance: All r-hFSH-α biosimilars analyzed presented differences compared to the originator. We firstly investigated Primapur and found significant differences regarding multiple structural attributes, particularly in the glycosylation profile. Gonal-*f* exhibited lower glycan branching, expressed by an A-index* of 2.5, while Primapur showed an A-index of 2.4. Furthermore, Primapur showed a lower level of sialylation in comparison with Gonal-*f*, as measured by their respective S-index* of 1.8 and 2.1. FSH glycosylation exhibits both macroheterogeneity and microheterogeneity, impacting both FSH protein's half-life and affinity with the follicle-stimulating hormone receptor (FSHR). Antennarity, representing FSH microheterogeneity, influence r-hFSH-activity since it has been shown that bulky and extended glycans may take longer to fit into the FSHR cavity compared to less sterically hindering glycans, resulting in a delayed response 7,8.Additionaly, sialylation has been shown *in-vivo* to correlate with plasma half-life and effect on granulosa cells proliferation 1,2,3. The slower clearance of highly sialylated r-hFSH has been shown to lead to a higher *in-vivo* activity, despite the lower *in-vitro* bioactivity 1,2,3.

**A-index and S-index express respectively a measure of the number of antennae and sialic acid per glycan. Final values are generated from many relative abundances normalized to 100, highlighting the significance of small numerical differences.*

Limitations, reasons for caution: More batches should be tested for each product. The authors are presenting full characterization of only one of the biosimilars since the rest of the products are under characterization.

Wider implications of the findings: r-hFSH-α originator and its biosimilar showed differences in terms of glycosylation profile that is well known as the major protein characteristic impacting FSH activity as extensively demonstrated in *in-vivo* and *in-vitro* models. This structural difference could have impact also on product efficacy and safety.

Trial registration number: 'not applicable'

P-662 Study on D-vitamin, AMH and insulin interrelation in Polycystic Ovary Syndrome (PCOS) patients.

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Study question: Is vitamin D deficiency more prevalent in PCOS patients? Is there a link between vitamin D levels and metabolic status in PCOS subjects?

Summary answer: An inverse relationship between vitamin D levels and metabolic status was demonstrated and it is thought to be responsible of its pathogenesis.

What is known already: PCOS is a multifactorial condition, characterised by failure in oogenesis and anovulation. Obesity is a common condition linked to its clinical features and studies have reported inverse associations between BMI and severity of the condition. Furthermore, 67–85% of PCOS patients have vitamin D deficiency.

Low levels of vitamin D have been found to be closely related to insulin resistance, obesity, or hyperandrogenism and there is a significant association between serum vitamin D levels and reproductive function.

Other factors such as AMH have also been described as possibly involved in the pathophysiology.

Study design, size, duration: We performed a retrospective, analytical and observational study in the Copenhagen Fertility Center. Patients referred with cycle abnormalities, hirsutism, and infertility were evaluated. A total of 778 women were enrolled consecutively from January 2019 to October 2020.

Subjects who had major medical disorders were excluded.

We selected those in which vitamin D was measured in the baseline analysis selecting a total of 396 patients.

The further analysis has been carried out from 100 randomly selected patients.

Participants/materials, setting, methods: Blood samples were drawn after overnight fasting. They were all assayed in the same laboratory.

Biochemical parameters were analyzed using descriptive statistics.

Same parameters were studied after dividing into vitamin D deficiency group or optimal levels using a multiple t-test.

Correlation between variables was determined.

Graphpad Prism program version 8 was used to perform the calculations.

The level of statistical significance was set at P-value < 0.05.

Main results and the role of chance: A total of 100 subjects fulfilling the inclusion criteria were selected randomly from 396 PCOS women.

Serum vitamin D concentrations were highly variable ranging from 16 nmol/L to 175 nmol/L.

The prevalence of vitamin D deficiency was 24% and 41% of the subjects were classified as vitamin D insufficient. Only 35% of our patients had optimal vitamin D values.

We compared data between the group with optimal values of vitamin D (Group A) versus the group with insufficient/deficient vitamin D values (Group B).

We found statistical difference between groups in PTH values, being notably higher in group B compared with group A. Despite no statistically significant difference was obtained, it is important to highlight that the mean of SHBG was lower in group B and the mean of androstenedione, AMH, FAI and HOMA-IR were much higher in this group as well.

Following the HOMA-IR criteria, 55% of patients had insulin resistance. Specifically, 26% had moderate insulin resistance and 29% severe insulin resistance. Levels of vitamin D were negatively correlated with FAI, AMH and HOMA-IR and positively correlated with HDL-Cholesterol and SHBG. Statistically significant differences were evidenced in the correlation between vitamin D and FAI and SHBG.

Limitations, reasons for caution: This is a retrospective observational study on a consecutive admitted patient group with a lack of a control group. Another limitation is the small sample size.

It is difficult to generalize with other degrees of severity. We didn't assess seasonal variability or if they were taking any vitamin D supplementation.

Wider implications of the findings: Properly randomized clinical trials are mandatory to achieve more conclusive results about the role of vitamin D. Available evidence is promising but not sufficient to draw final conclusions. The aim is to better understand the pathophysiology of the condition and the factors involved and to find new target treatments.

Trial registration number: I

P-663 The ratio of serum progesterone (P4) to the number of follicles (P4/Follicle) is a more objective parameter for euploidy rate as compared to systemic progesterone

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Study question: Does the ratio of serum progesterone (P4) to the number of follicles (P4/Follicle) on the day of final oocyte maturation affect the ploidy status of the embryos?

Summary answer: A high P4/Follicle ratio negatively affects the euploid rate of the embryos.

What is known already: During ovarian stimulation, exogenous gonadotropins are administered to achieve multifollicular growth. Intense gonadotropin stimulation towards the end of the follicular phase seems to cause a premature progesterone rise in stimulated IVF cycles. The impact of serum progesterone elevation during the follicular phase has been studied intensively. Though most studies have focused on the effect of progesterone elevation on the endometrial receptivity, little is known about its possible impact on embryo development and ploidy status. The only study that investigated the effect of progesterone on the embryo ploidy status, was unable to show any significant impact.

Study design, size, duration: This retrospective study was performed at ART Fertility Clinics Abu Dhabi, UAE and Muscat, Oman. All stimulation cycles (n=975) were performed between January 2015 to December 2019 with patients aged between 18-45, Body mass index (BMI) of 18-35, stimulated either with rFSH or hMG. All embryos underwent ICSI and Preimplantation Genetic Testing for Aneuploidies (PGT-A),Patients with surgical sperm extraction, warmed oocytes or natural cycle IVF were excluded.

Participants/materials, setting, methods: Serum P4 was measured on the last ultrasound prior triggering for final oocyte maturation. The P4/Follicle ratio was calculated as the ratio of P4 on trigger day to the number of follicles > 10 mm on the last ultrasound.

Serum P4 and P4/Follicle ratio were then analyzed using linear and univariate regression model to find potential correlation with the number of oocytes retrieved, number of mature oocytes, embryo quality (day 3 and 5), and euploid rate.

Main results and the role of chance: A total of 975 cycles were analyzed, with a mean age of 33.88±0.05 years, a mean BMI of 26.7±0.035 kg/m2. The mean number of oocytes collected was 12.53±0.058.

Mean serum P4 on trigger day was 0.83±0.005 ng/ml and higher serum P4 values were observed as the number of oocytes retrieved and the number of mature oocytes increased (β=0.026, p<0.0001 and β=0.028, p<0.001, respectively). On the other hand, the mean P4/Follicle ratio was 0.056±0.00041 ng/ml and, unlike serum P4, the P4/Follicle ratio showed a negative correlation with the number of oocytes retrieved as well as with the number of mature oocytes (β=-0.001, p<0.001 and β=-0.001, p<0.001, respectively).

While day 3 embryos were not affected by serum P4 or P4/Follicle ratio, the blastocyst quality was negatively affected by both increasing serum P4 levels and the P4/Follicle ratio (β=-0.012 p<0.05, β=-0.002, p<0.001, respectively).

Euploid rates were positively correlated in cycles with increased serum P4 β=0.18, p<0.001), while negatively correlated in cycles with a high P4/Follicle ratio (β=-0.015, p<0.001).

After adjusting for potential confounders, only P4/Follicle remained as a significant negative factor for euploid rate (β=-0.004, p<0.001, 95% CI: -0.007--0.001, p<0.001), which was not observed for serum P4 (p=0.46).

Limitations, reasons for caution: This is an observational study based on retrospective data; an improved extrapolation of the results might be obtained by performing a prospective study.

Wider implications of the findings: The findings of this study should encourage clinicians to optimize the ovarian stimulation protocols not only based on serum P4, but also considering the P4/Follicle ratio.

Trial registration number: Not applicable

P-664 Bone morphogenetic protein-7 (BMP-7) reduces E2 and P4 production of human luteinized granulosa cells by down-regulating the expression of the steroidogenic enzymes StAR and 3B-HSD

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Study question: What is the biological role of BMP-7 on the granulosa cells after luteinization?

Summary answer: BMP-7 down regulates the steroidogenic enzymes and reduces E2 and P4 output of luteinized granulosa cells.

What is known already: BMP-7 is a member of TGF-B super family that is mainly produced by theca cells in the ovary. It promotes the transition of primordials into primary follicles, and the growth and preantral and antral follicles, and inhibits progesterone (P4) production during FSH-induced growth phase of Graafian follicles (luteinization inhibitor). However, limited data is available regarding the role of this hormone on the molecular luteal characteristics of granulosa cells after ovulation and luteinization processes. We therefore aimed to address this issue in the current study.

Study design, size, duration: A basic science study on the corpus luteum biology

Participants/materials, setting, methods: Human luteal granulosa cells were obtained from 10 normo-responder IVF patients undergoing ovarian stimulation with rec-FSH and GnRH antagonist protocol and cultured with recombinant forms of BMP-7, hCG and activin-A for 24h. The presence of cognate receptors for these hormones were validated using RT-PCR. The expression of the steroidogenic enzymes were analyzed with quantitative immunoblotting, real-time RT-PCR and confocal microscopy. E2 and P4 production of the cells were measured using ECLIA method.

Main results and the role of chance: BMP-7 significantly down-regulated the expression of StAR and 3b-HSD in immunoblotting and confocal images and caused a substantial decrease in P4 production in the luteal GCs in a dose dependent manner. It did not cause any notable change in aromatase expression, however E2 output declined in parallel with P4 due to the reduced expression of StAR, which is the rate limiting enzyme in steroidogenesis. hCG significantly up-regulated StAR and 3b-HSD expression and enhanced P4 output whereas activin-A did the opposite effect. Viability assay with Yo-PRO-1 uptake assay did not reveal any significant differences in the viability of the cells before and after treatment with these hormones.

Limitations, reasons for caution: In-vitro findings requires validation using in-vivo models.

Wider implications of the findings: Reversal of luteinization and down-regulation of steroidogenesis with BMP-7 and other hormones with similar actions warrant further investigation to test their in-vivo effects in order to develop new strategies against ovarian hyperstimulation syndrome (OHSS).

Trial registration number: not applicable

P-665 Influence of premature progesterone elevation on embryo development

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Study question: Does a serum progesterone level higher than 1.3 ng/mL on the day of ovulation trigger have an impact on blastocyst development?
Summary answer: Elevated progesterone level has no significant impact on top blastocyst rate, usable blastocyst rate and on morphokinetics.
What is known already: Premature elevation of progesterone level on the day of ovulation trigger prior to IVF is common and causes a decrease in endometrial receptivity. A freeze all strategy is then recommended. However, cumulative live birth rates have also been described as lower in cases of high progesterone levels.
Study design, size, duration: This was a retrospective bicentric cohort follow-up study, including 1150 IVF/ICSI cycles performed between 2016 and 2018 with at least 1 day-5 blastocyst available for transfer or freezing. Among these cycles, 524 were performed with use of a time-lapse system (Embryoscope). Serum Progesterone level was measured on the day of ovulation trigger, and a value >1.3 ng/ml was used to identify premature progesterone elevation.
Participants/materials, setting, methods: The cycles were divided into 2 groups according to serum progesterone level: 1335 cycles were allocated in the normal progesterone group (P<1,3) and 215 in the progesterone premature elevation group (P>1.3). Patient's characteristics, ovarian stimulation characteristics, IVF cycles characteristics and embryology parameters were anonymously recorded and compared between the 2 groups.
Main results and the role of chance: Female age, smoking status, AFC and AMH levels were comparable between the 2 groups. Female BMI was significantly higher in the P<1,3 than in the P>1.3 group (26.1 versus 24.7 kg/m² respectively). Total FSH dose, estradiol level, number of follicles >11mm and number of retrieved oocytes were significantly higher in the P>1.3 group than in P<1.3 group No difference was observed between the 2 groups in terms of top blastocyst rate per mature oocyte and usable blastocyst rate per mature oocyte. When morphokinetic analysis was available, time to blastulation was the only significantly different parameter between the 2 groups (110.4 hours in P<1.3 versus 107.9 hours in P>1.3, p=0.04). Cumulative live birth rate per cycle was not statistically different between the two groups (23.1% for P<1.3 versus 28.7% for P>1.3) (p > 0.05).
Limitations, reasons for caution: The retrospective design of the study should lead to careful analysis of the results. The progesterone threshold refers to a specific assay, and should not be generalized to other assays.
Wider implications of the findings: Premature elevation of serum progesterone level on the day of ovulation trigger does not seem to affect embryo developmental competence. This further supports the relevance of freeze all strategy in this situation.
Trial registration number: not applicable

P-666 Validating the hypo-androgenic PCOS-like phenotype (H-PCOS), derived from the "lean" PCOS phenotype at younger ages

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Study question: Is the resistance to standard infertility treatments of the H-PCOS-like phenotype reversed through reconstitution of androgen levels and can principle diagnostic markers of H-PCOS be validated?
Summary answer: Pre-supplementation with dehydroepiandrosterone (DHEA) eliminated treatment resistance of H-PCOS in comparison to matched infertile controls, also validating previously reported diagnostic features of this condition.
What is known already: H-PCOS evolves at older ages from a hyper-androgenic "lean" PCOS phenotype at young ages. Its ontogeny diverts from other PCOS phenotype between 20s and mid-30s by going from being hyper- to being hypo-androgenic due to insufficiency in adrenal androgen production, believed to represent an autoimmune process. In contrast to other PCOS phenotypes, the "lean" PCOS phenotype appears highly treatment resistant to standard fertility treatments.
Study design, size, duration: Retrospective case control study.
Participants/materials, setting, methods: We investigated 54 H-PCOS patients with qualifying diagnostic criteria1,2 and 50 matched infertility patients without diagnostic H-PCOS criteria as controls. Both study groups underwent routine in vitro fertilization (IVF) cycles, including androgen pre-supplementation in both groups via dehydroepiandrosterone (DHEA) for women diagnosed as hypo-androgenic. Main outcome measures were clinical pregnancy and live birth rates.

[1]Gleicher et al., J Sterodi Biochem Mol Biol 2017;167:144-152; 2Gleicher N, et al., Endocrine 2018;59(3):661-676

Main results and the role of chance: Study groups were similar in age, number of prior IVF cycles and previous live births. H-PCOS patients in contrast to controls, however, demonstrated previously reported characteristics of H-PCOS diagnosis, including a significantly higher DHEA/DHEAS ratio, significantly higher AMH, confirming higher functional ovarian reserve, significantly lower free testosterone and significantly higher sex hormone binding globulin (SHBG), further confirming lower androgens. Finally, H-PCOS patients also demonstrated significantly increased evidence for immune system hyperactivity. Clinical pregnancy and live birth rates were separately assessed in first IVF cycles and cumulatively. Both analyses demonstrated, even after age-adjustments, absolutely no outcome differences in cycle cancellations, numbers of oocytes retrieved, first and cumulative pregnancy and live birth rates. At least one pregnancy was achieved in 12 women in both groups (22.2% and 24.0%) and at least one live birth in 11 (20.4%) vs. 8 (14.8%), respectively.
Limitations, reasons for caution: As a retrospective case control study, here presented data must be interpreted with caution. The close match between H-PCOS and control patients and the very clear differentiation in patient characteristics between the two groups, however, support the credibility of this study.
Wider implications of the findings: This study demonstrated that androgen reconstitution in H-PCOS patients completely reversed treatment resistance compared to well-matched infertile control patients. It also validated previously defined diagnostic criteria of H-PCOS, hopefully facilitating a timelier diagnosis of a, still, widely overlooked condition in female infertility.
Trial registration number: NA

P-667 Dydrogesterone supplementation in cycles triggered with lone GnRH agonist for final oocyte maturation resulted in an acceptable pregnancy rate

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Study question: Can luteal oral Dydrogesterone (Duphaston) supplementation in an antagonist cycle after a lone GnRH agonist trigger rescue the luteal phase, allowing the possibility to peruse with fresh embryo transfer?
Summary answer: Functionality of the luteal phase in an antagonist cycle after a lone GnRH agonist trigger can be restored by adding Duphaston to conventional luteal support.
What is known already: Ovarian hyperstimulation syndrome (OHSS) is dramatically reduced when using antagonist cycle with lone GnRH agonist trigger before ovum pick up. This trigger induces short luteinizing hormone (LH) and follicle-stimulating hormone (FSH) peaks, associated with reduced progesterone and estrogen levels during the luteal phase. They cause an inadequate luteal phase and a significantly reduced implantation rate leading to a freeze all practice in those cycles.

Study design, size, duration: A retrospective cohort study. The study group (n=123) included women that underwent in vitro fertilization cycles from January 2017 to May 2020. Patients received a GnRH-antagonist with a lone GnRH-agonist trigger due to imminent OSHH. The control group (n=374) included patients under 35 years old that, during the same time period, underwent a standard antagonist protocol with a dual trigger of a GnRH-agonist and hCG.
Participants/materials, setting, methods: Study patients were given Dydrogesterone (Duphaston) in addition to micronized progesterone vaginal pills (Utrogestan) for luteal support (Duphaston group). Controls were treated conventionally with Utrogestan for luteal phase support (hCG group). The outcomes measured were pregnancy rate and OHSS events.
Main results and the role of chance: Our study was the first to evaluate the addition of Duphaston to standard luteal phase support in an antagonist cycle triggered by a lone GnRH agonist before a fresh embryo transfer. The mean number of oocytes retrieved and estradiol plasma levels were significantly higher in the Duphaston group than in the hCG group (16.9 ±7.7 vs. 10.8 ± 5.3 and 11658 ± 5280 pmol/L vs. 6048 ± 3059 pmol/L, respectively). The fertilization rate was comparable between the two groups. The mean number of embryos transferred and the clinical pregnancy rate were also comparable between groups (1.5 ± 0.6 vs 1.5 ± 0.5 and 46.3% vs 40.9%, respectively). No OHSS event was reported in either group.
Limitations, reasons for caution: This retrospective study may carry an inherent selection and information bias, derived from medical record coding. An additional limitation was the choice of physician for the lone GnRH trigger, which may have introduced a selection bias and another potential caveat was the relatively small sample size of our study groups.
Wider implications of the findings: The addition of Duphaston to conventional luteal support could effectively salvage the luteal phase without increasing the risk for OHSS. This enables, to peruse in those cycle, with fresh embryo transfer, avoiding the need to freeze all the embryos and postponed embryo transfer. Leading to lower psychological burden and costs.
Trial registration number: 0632-20-HMO

P-668 We aim for one baby, not one embryo: a personalized ET strategy based on embryo score and woman age maximizes LB and minimizes twins

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Study question: Is it possible to define a personalized ET model to maximize the chance of live birth (LB) while minimizing the risk of twin pregnancy?
Summary answer: A model including age and embryo morphological score can inform a personalized ET strategy to maximize LB while minimizing the risk of twin pregnancy.
What is known already: The morphological score of the transferred embryos affects pregnancy (PR) and LB rates in IVF cycles. Although SET is mainly recommended to avoid multiple pregnancies, DET is still being performed extensively, especially in patients with poor prognosis, with the aim to improve PR per transfer and shorten time to pregnancy. While twin pregnancies are associated with an increased risk of maternal and fetal complications, very low PR may increase patient drop-off, extend time to pregnancy, and increase the cost per successful transfer. A personalized transfer strategy balancing high LB per transfer with low twin pregnancy rates should be defined.
Study design, size, duration: Retrospective study including 2,470 fresh and frozen embryo transfers (ET) of either one or two embryos at D3 from January 2016 to August 2019 in a single IVF clinic. Biochemical, clinical, multiple pregnancy and live birth rates after SET and DET were analyzed according to the morphological score of the embryos transferred. ETs were divided into 9 groups according to the combinations of their embryo morphological scores.
Participants/materials, setting, methods: Embryos were assessed on D3 following a national recommended morphological scale. Morphology was categorized as High (H) if A or B+, medium (M) if B or C+, and Low (L) if C or D. The likelihood of biochemical, clinical pregnancy and live birth, and the risk of multiple pregnancy, after SET and DET of embryos of different scores was analyzed. A logistic regression analysis adjusted by age of the woman was ran for each outcome.
Main results and the role of chance: The distribution of ETs among the 9 groups for SET was: 510 H, 715 M, 346 L; for DET: 142 HH, 148 HM, 29 HL, 268 MM, 164 ML, 148 LL. Mean woman age was similar among groups: 38.7±4.01. Live birth and twin rates increased with embryo score. Considering a SET of category M as reference, the OR of live birth in DET were: 2.76 [1.82, 4.19 95%CI] for HH, and 2.32 [1.51, 3.55 95%CI] for HM, and 1.69 [1.19, 2.40 95%CI] for MM, and in SET: 1.52 [1.12, 2.04 95%CI] for H. Considering a DET of category MM as reference, the OR of twin birth in DET were: 2.8 [1.14, 6.99 95%CI] for HH, 2.5 [0.98, 6.46 95%CI] for HM, and 0.92 [0.11, 7.84 95%CI] for HL. According to this model, a 38y.o. woman with a SET of category M would have a 16% chance of live birth, and no twins. The addition of an M (DET: MM) increases her chance of live birth to 24% with a 2.9% risk of twins. The addition of a H (DET:MH) would increase further her chance of live birth to 30.8%, however, the increase would be due almost exclusively to twins (7%).
Limitations, reasons for caution: The limitations of this study are its retrospective nature and the small size of some categories. Embryos were classified using a national morphological scale; other morphological classifications could influence the results. The development and validation of site-specific models, using local patients' data, is recommended before their use in clinical practice.
Wider implications of the findings: A personalized assessment of embryo quality and woman age, at a minimum, are necessary to identify the best ET strategy for each patient; this strategy allows to maximize live birth rates while keeping the risk of twin birth as low as possibl.
Trial registration number: not applicable

P-669 "Follitropin": A retrospective, observational study comparing the efficacy of follitropin alpha biosimilar therapy in different ovarian stimulation protocols: real-world data

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Study question: To investigate the therapeutic efficacy of follitropin alpha biosimilar therapy in nonselected patients undergoing IVF.
Summary answer: This large retrospective study demonstrated similar therapeutic efficacy for follitropin alpha biosimilar therapy in women who underwent ovarian stimulation (OS) using different protocols.
What is known already: Based on data from the last meta-analyses (Budani et al., 2020), follitropin alpha biosimilars showed similar efficacy and safety in randomized controlled trials aimed at proving the therapeutic equivalence in terms of oocytes retrieved in women undergoing OS. In most cases, normogonadotrophic patients were enrolled in such studies without any endocrine or ovarian disturbances. The absence of real-world data can be compensated by additional post-marketing studies aimed at investigating the efficacy of biosimilars in different OS protocols using antagonists and agonists of GnRH and OS with mixed gonadotropins.
Study design, size, duration: A retrospective, observational, anonymized cohort study conducted at 35 IVF clinics in Russia, named "FOLLITROPIN", compared the efficacy of OS in 2020. The OS protocols analysed where follitropin alpha biosimilar (Primapur®) was applied for at least 5 days. All of the analysed subjects underwent OS using GnRH antagonist/agonist protocols, with

no restrictions on the OS protocol or food supplements/vitamins. No inclusion or exclusion criteria were applied. Overall, 5484 OS protocols were analysed.
Participants/materials, setting, methods: The efficacy of 5484 OS protocols was calculated, and two subgroups were extracted: (1) mixed gonadotropin OS protocols (N=2625) *vs* monotherapy with Primapur® (N=2859); (2) GnRH antagonist OS (N=2183) *vs* GnRH agonist (N=676) using only Primapur®. Demographic and clinical characteristics: (1) Age 34.9±4.8 *vs* 32.9±4.7 (*p<0.001*), BMI 23.9±4.7 *vs* 23.6±4.5 (*p<0.001*), IVF attempt 1.4±0.7 *vs* 1.3±0.6 (*p<0.001*); (2) Age 32.9±4.6 *vs* 33.1±4.9 (*p=0.449*), BMI 23.7±4.6 *vs* 23.1±4.5 (*p=0.019*), and IVF attempt 1.2±0.5 *vs* 1.4±0.9 (*p<0.001*).
Main results and the role of chance: The total efficacy of OS with Primapur®: oocytes retrieved: 9.5±7.2, MII: 6.8±6.6, 2PN: 6.1±5.8, clinical pregnancy per ET (PR) 6 weeks after ET: 38.4%. Subgroup 1 analysis: oocytes retrieved: 8.6±6.8 *vs* 10.3±7.4 (*p<0.001*), MII: 6.7±6.2 *vs* 7.7±6.9 (*p<0.001*), 2PN: 5.8±5.2 *vs* 7.2±6.2 (*p<0.001*). There were statistically significant differences between oocyte yields in mixed *vs* monotherapy protocols due to subgroup differences, including age, BMI and IVF attempts. No statistically significant differences were found for PR in subgroup 1: 39.3% [95% CI: 36.9-41.7%] *vs* 37.6% [95% CI: 35.3-39.8%] (*p=0.314*). The major accompanying gonadotropin in the mixed protocol was menotropin (75% for mixed OS protocols). Subgroup 2 analysis: oocytes retrieved: 10.5±7.5 *vs* 9.6±7.0 (*p=0.032*), MII: 7.6±6.9 *vs* 6.7±5.7 (*p<0.001*), 2PN: 7.3±6.3 *vs* 5.7±5.0 (*p<0.001*). There were statistically significant differences between oocyte yields in GnRH antagonist *vs* GnRH agonist monotherapy due to subgroup differences, including BMI and IVF attempts. No statistically significant differences were found for PR in subgroup 2: 37.9% [95% CI: 35.5-40.5%] *vs* 35.9% [95% CI: 30.8-41.1%] (*p=0.482*). All medicines were well tolerated at the injection site, and no serious adverse reactions were reported. This large retrospective cohort study in a nonselected population demonstrated similar clinical efficacy for follitropin alpha biosimilar therapy.
Limitations, reasons for caution: The real-world patient data analysed in this study were representative, showing the ability of follitropin biosimilars to develop both folliculogenesis and clinical pregnancy in a nonselected population. Additional comparative studies are needed to confirm the efficacy of the biosimilars in patients with classified types of infertility causes, including unexplained infertility.
Wider implications of the findings: In this study, we demonstrated the therapeutic efficacy of biosimilars in terms of oocyte yield and clinical pregnancy development in women undergoing different OS protocols. Further large-scale studies with known hormonal levels before and during OS, as well as the micro- and macronutrient status of both parents, are needed.
Trial registration number: None

P-670 Urine estrone-3-glucuronide (E3G) assay: is there any place during ovarian stimulation for IVF cycles?

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Study question: Could the urine estrone-3-glucuronide (E3G) assay be used efficiently to monitor a controlled ovarian hyperstimulation (COH) cycle, in comparison to a serum estradiol (E2) assay?
Summary answer: E3G testing provides an alternative to serum E2 assessment and a new "patient friendly" approach for COH monitoring.
What is known already: In many IVF clinics basic monitoring tools for controlled ovarian stimulation during IVF procedure are ultrasound measurements of follicle growth and hormone assessment of serum E2 levels. The monitoring can occur 4-6 times during stimulation, but repeated blood sampling causes patient stress. In contrast, E3G sampling, one of principal metabolites of estradiol in urine, is non-invasive and can be performed by the patients themselves and measured by fluorescent immunoassay. A correlation has been shown between concentrations of E2 present in plasma and concentrations of E3G in different phases of menstruation cycle.
Study design, size, duration: This is a pilot, prospective study, in a single IVF clinic. Twenty female participants were recruited November 2020 -January 2021, aged 25-43 years and BMI: 18-28kg/m2. Dynamic change of serum E2 and urine E3G at ovarian stimulation monitoring are being analyzed.
Participants/materials, setting, methods: Concurrent urine E3G and serum E2 values were collected from patients who provided between 2 and 4 samples on different days of their COH IVF cycle. Serum E2 values were assessed routinely, while E3G values were measured and validated using a fluorescent immunoassay Mira Fertility Plus® analyzer.Main results and the role of chance: The urine E3G of assay was validated for intra- and inter-assay variability with a coefficient of variation of<20%. It was also validated for analytical and functional sensitivity and sample stability. Linear regression of serum E2 and E3G values of 56 early morning urine samples who had evaluated between Days 4 and 13 of menstruation cycle provided an R=0,81. Urine E3G values also correlated to follicle growth. Patient survey results showed that urine sampling was the preferred method of analysis.
Limitations, reasons for caution: We have provided proof of principle that urine E3G measurement can be accurately carried out using fluorescent immunoassay technology during routine COH for IVF cycles. The patients' study group has to be expanded in order to enable us to find the appropriate place of urine E3G assay in COH protocol.
Wider implications of the findings: Urine E3G testing correlates well to serum E2 assessment in COH. Urine E3G assay provides an alternative to serum-based assessment. The ease of urine sampling allows a reduction in patient discomfort during venopuncture, costs, time, and infection risks in epidemics/pandemics, like COViD-19, and offers a patient-friendly approach to ovarian stimulation.
Trial registration number: NA

P-671 Dual Trigger(low adjuvant HCG4h after GnRHagonist trigger)have positive impact of overall embryo's quality, implantation ,clinical pregnancy and live birth rates in high-risk patients for OHSS

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Study question: Study aim is to compare implantation,clinical pregnancy and livebirth rates between giving1500IU of hCG4hours after GnRHagonist,on trigger day or GnRHagonist as alone trigger with luteal support withHCG1500IU.35h later on OPUday.
Summary answer: Adjuvant doze of1500IUhCG4h after bolus of GnRHagonist on trigger day significantly improve quality of blastocyst,implantation,clinical pregnancy and live birth rates without increasing the risk ofOHSS.
What is known already: The use of GnRHagonist for final oocyte maturation in antagonist cycle significantly decrease the incidence of OHSS,but there have been studies showing lower pregnancy rates in patients triggered with GnRHagonist compared with hCG in autologous cycles,attributed to a defective luteal phase, especially in high–risk patients despite intensive luteal phase support.To improve the results of IVF,an alternative approach is adding a small bolus dose of hCG(1500IU)35h later,on the OPU day after GnRHagonist trigger which provides more sustained support for the corpus luteum.The question is does low doses of hCGgiven on the same day with GnRHagonist trigger is making better quality oocytes.
Study design, size, duration: Single center prospective longitudinal cohort study fromJanuary2017 to Decembar2019.The initial inclusion criteria were:women age≥18and≤39years,AMH≥3,3ng/ml and ≥12 antral follicles on basal ultrasound. Patients with history of OHSS and PCO are also included in the study.Patients with applied "freeze-all" technique with peak estradiol≥4000pg/ml on trigger day>18oocytes on the OPU day,and recognized significant risk for developing OHSS were also included.The cumulative implantation,clinical pregnancy and live birth rates were analyzed,only in embryos from the same COS protocol in every patient.
Participants/materials, setting, methods: A total of 231 patients were entered for final analysis,who underwent a flexible antagonist protocol,ICSI and fresh or thawed ET on 3th(38.53%) or 5th(61.47%)day in women's autologous cycles.Patients were randomized in one of two groups: GroupA-Dual trigger group 1500IUof hCG 4h after GnRH agonist application on trigger day and GroupB -1500IU of HCG 35h later,on the OPU day.We used nonparametric and parametric statistical tests.Significant differences were considered all values of p <0.05
Main results and the role of chance: Both groups are homogenous regarding several variables:age,BMI,type of sterility,smoking status,AMH,PCO, spermogram.There is no significant difference between the two(AvsB)groups according to average number of retrieved oocytes(13.6 vs 14.6 p>0,05),M II oocytes(11.03 vs 11.99 p>0.05).The dual trigger group(A)had a higher fertility

rate(69.99% vs 64.11% p<0,05)compared with GnRHagonist trigger group(B). There are no significant difference between groups(AvsB)according to cumulative average number of:transferred embryos(2.4vs2.5 p>0.05)TQE transfered on 3th day(1.5.vs 1.3.p>0.05);transferred blastocyst(2.6 vs2.7 >0.05);cryo embryos(2.5vs1.9 p>0.05),but there are significant difference according to cumulative implantation rate of transferred blastocyst in favor of group A(48.18% vs 33.89%p<0.05).Analyzes of morphological characteristics of transferred blastocyst depicted in the order of degree of blastocyst expansion,inner cellular mass(ICM)and trofoectoderm(TE) and ranking overall blastocysts quality from"-excellent","good","average" and "pore" ,shows that there are significantly more percentage of patient with embryo transfer of "excellent" or even one "excellent" blastocyst in group A (30.56%,31.94% vs 21.54%,23.08% p<0.05) in opposite of percentage of patients with embryo transfer with "poore "" blastocyst in group B (37.5 % vs 46.15.%p<0.05). Clinical pregnanacy rate (71.68 % vs 50.84% p< 0.05) , and live birth rate (60,18 % vs 42,58%), were significantly higher in group A. There were no cases of moderate or severe OHSS in both groups.

Limitations, reasons for caution: Dual trigger in GnRH antagonist protocols should be advocated as a safe approach but undetected high risk patients are reasons for caution for developing clinically significant OHSS.

Wider implications of the findings: Adjuvant low dose of hCG on GnRHagonist trigger day improve clinical pregnancy and live birth rates without increasing the risk of clinically significant OHSS.Protocol of dual trigger and freezing all oocytes or embryos in patients with high risk of developing OHSS is promising technique in everyday practice.

Trial registration number: 8698

P-672 Higher pregnancy outcomes in patients undergoing embryo transfer-under hormonal replacement therapy where an individualised Progesterone supplementation was applied on the day of β-hCG

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Study question: Does progesterone-supplementation (PS) from the day of β-hCG assessment improve pregnancy rates in embryo transfer-under hormonal replacement therapy (ET-HRT) in patient with Progesterone (P)<10.6 ng/mL?

Summary answer: Reduced P on the β-hCG day is associated with lower pregnancy-rates and higher miscarriage-rate. PS from the same day showed significant increase of reproductive outcomes.

What is known already: Up until now, in ART, very little has been done to understand whether the P intake should be personalized during the luteal phase. Most recent studies on the topic showed that low P levels on the day of ET-HRT or on the day before are associated with decreased pregnancy rates; however, when low P values are supplemented from the day before embryo-transfer (ET), similar results to cases with adequate P are reported. Nevertheless, little is known about the association between low P level, on the day of β-hCG (P-β-hCG) and PS from this day in ET-HRT, and pregnancy outcomes.

Study design, size, duration: This is a single centre, cohort, retrospective study conducted at a university-affiliated fertility centre between January 2018 and June 2020 where PS took place from the day of positive β-hCG determination when P <10.6 ng/mL. In total 789 ET-HRT cycles were analysed of which 239 were performed in both fresh and frozen heterologous ET-HRT (het-ET), 336 in homologous ET-HRT (hom-FET) and 214 in euploid ET-HRT (eu-FET) after preimplantation genetic testing for aneuploidies IVF cycles (PGT-A).

Participants/materials, setting, methods: Women undergoing ET-HRT with normal P (>10.6ng/mL) on the day before ET were screened for P on the day of β-hCG. All women received vaginal P 200 mg/8 hours for the second part of HRT. PS was performed by adding P to the HRT when P-β-hCG was considered low (<10.6 ng/mL). Primary outcome: ongoing-pregnancy-rate (OPR); secondary outcome: miscarriage-rate (MR). Both were evaluated by considering PS on the day of β-hCG as a categorical variable.

Main results and the role of chance: Patients characteristics were comparable between groups (het-ET, hom-FET and eu-FET) although significantly lower body mass index was found when P-β-hCG>10.6 ng/mL compared to the subgroup with P-β-hCG<10.6 ng/mL and no PS (p=0.012). Overall clinical pregnancy rate was 52.1% with no-significant differences between groups (48.5% in het-ET, 52.9% in hom-FET and 54.7% in eu-FET). P-β-hCG was considered as adequate in 75.7% (311/411) ET-HRT with positive β-hCG and low in 24.3% (100/411), with no differences between groups. In case of positive β-hCG and P-β-hCG >10.6 ng/mL, OPR was 83.6% and MR was 16.4%, with no-significant differences between groups. Among the 100 low P-β-hCG, 80 ET-HRT received PS. In this subgroup OPR was 96.2% and MR was 3.8%, with no-significant differences between groups. In 20 out of 100 ET with P-β-hCG <10.6 ng/mL, no PS was added for different reasons. This group showed the lowest OPR (30%) and the highest MR (70%), again with no between-group differences according to het-ET, hom-FET or eu-FET. Miscarriage rate was significantly higher (p<0.001) when P-β-hCG was <10.6 ng/mL and no PS was added to HRT compared to P-β-hCG <10.6 ng/mL but with PS, and also compared to the P-β-hCG >10.6 ng/mL group.

Limitations, reasons for caution: The main limitation of the study is due to its retrospective nature and the small sample of patients with P-β-hCG<10.6 ng/mL that was not supplemented. Furthermore, the cut-off of P-β-hCG was arbitrarily decided upon previous studies, and lastly different routes of administration were considered for the PS.

Wider implications of the findings: The results of this study showed that individualization of Progesterone supplementation in ET-HRT may be a crucial turn point in order to increase the pregnancy rates and decrease the miscarriage rates. An adequate PS should be considered in case of low P-β-hCG levels for both het-ET, hom-FET and eu-FET.

Trial registration number: Not applicable

P-673 A visualized clinical model predicting cumulative pregnancy rate after IVF-ET: a real-world study

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Study question: How is the cumulative pregnancy probability of individual patients after IVF-ET,could we develop a visualized clinical model to predict it based on patient's characteristics?

Summary answer: The visualized clinical mode incorporates five items of female age, number of oocytes, antral follicle count, endometrium thickness and basal FSH level.

What is known already: Many factors can result in infertility, prognosis prediction is clinically relevant for making the right therapeutic strategy while avoiding overtreatment. It is also helpful in counselling, making the patient aware of possible treatment duration and estimated expense and managing patient's expectation. Visualized clinical mode and accurate prediction would also be helpful in designing clinical trials to evaluate new treatments.

Study design, size, duration: We conducted a retrospective analysis of a single-center database using prospectively collected data from women who underwent IVF/ICSI treatment from January 2013 to December 2015, All the participants were followed up for at least 2 years, 3538 IVF-ET cycles were included in the study.A total of 3538 IVF/ICSI cycles were included in the study.

Participants/materials, setting, methods: Data from a total of 2312 IVF/ICSI cycles from January 2013 to December 2014 were randomly split into training dataset (1550, 67%) and internal validation dataset (762, 33%). A total of 1226 IVF/ICSI cycles in 2015 was applied to external validation dataset (temporal validation)

Main results and the role of chance: Multivariable logistic regression model combined with restricted cubic splines function was used to test independent prognostic factors and estimate their effects on treatment outcome for patients treated with IVF/ICSI. Female age, number of oocytes retrieved, AFC, endometrium thickness and basal FSH were included the final model. The above model was used to calculate prediction scores for all women in the training and validation datasets. The C-index was 0.693 (95% CI: 0.692~0.695) in training sets, 0.689 in internal validation sets and 0.710 in external validation sets, which denotes a good performance. Calibration curves suggest excellent model calibration, with an ideal agreement between the prediction and actual observation . The DCA showed that if the threshold probability is between 0 and 0.7, using

the nomogram derived in the present study to predict cumulative pregnancy provided a greater benefit than either thetreat-all or the treat-none strategy.
Limitations, reasons for caution: it was a retrospective, single-center study. In the future, prospective, randomized controlled, multicenter clinical studies will be designed.
Wider implications of the findings: The visualized nomogram model provides great predictive value for infertility patients in their first IVF/ICSI cycle, and predicts the pregnancy probability of individuals ,and could help clinicians improving clinical counselling.
Trial registration number: not applicable

P-674 Development of a predictive model indicating the population of poor responders benefiting from luteal phase oocyte retrieval

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Study question: Can successful implementation of luteal phase oocyte retrieval (LuPOR) following conventional follicular phase oocyte retrieval (FoPOR) be predicted for poor ovarian response (POR) patients?
Summary answer: Antral follicle count (AFC), number of small follicles recorded in FoPOR, and estradiol (E2) levels on FoPOR and LuPOR trigger days, predict successful LuPOR application.
What is known already: A second follicular wave in the same menstrual cycle was first observed in domestic animals such as horses and cattle and thenceforth in women. The second follicular wave has been introduced as an encouraging means towards optimizing the context of in vitro fertilization (IVF) success rates for infertile women and especially for POR patients. Double ovarian stimulation coupled with two oocyte retrievals in the same menstrual cycle has been proposed, and encouraging results have been reported. However, the high heterogeneity characterizing POR patients dictates that studies should focus on factors indicating efficient LuPOR application.
Study design, size, duration: This retrospective observational study included 1688 women diagnosed with POR, undergoing natural IVF cycles between 2012-2020 including two oocyte retrievals in the same menstrual cycle. Patients' age, body mass index (BMI), number of previous POR incidences, basal hormonal levels, AFC, E2 evaluated on both trigger days and number of small follicles (8-13 mm) were evaluated on their predictive power regarding retrieval of at least one MII oocyte following LuPOR, being regarded as successful LuPOR implementation.
Participants/materials, setting, methods: A diagnosis of POR according to Bologna criteria served as the inclusion criterion for this single center study. All other infertility etiologies were excluded. Patient dataset was stratified according to age in quantiles. A random 20% of each quantile was employed to validate the model. The remaining 80% was employed to develop this model. The predictive value was determined employing the Area Under the Curve (AUC) of the Receiver Operating Characteristics, employing Youden's index**.**
Main results and the role of chance: Patients' age, BMI, number of previous failed IVF attempts, basal levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin and progesterone failed to be predictive of a successful LuPOR as the AUC was below 0.6. AFC with a threshold value of 4.47, was found to be predictive of an effective LuPOR with an AUC of 0.86, sensitivity 0.8, specificity 0.75, and accuracy 0.79. E2 levels evaluated on the FoPOR trigger day, with a threshold value of 232.66 pg/ml, were similarly predictive of an effective LuPOR presenting with an AUC 0.86, specificity 0.75, sensitivity 0.86 and accuracy 0.82. Similarly, E2 evaluated on the LuPOR trigger day, with a threshold value of 200.89 pg/ml, presented with an AUC 0.89, specificity 0.85, sensitivity 0.95 and accuracy 0.92. The number of small follicles during FoPOR also appeared to be predictive of the presence of at least one MII oocyte during LuPOR, with a threshold value of 2.94. The AUC was 0.82, specificity 0.75, sensitivity 0.76 and accuracy 0.75. When combining the above characteristics into a single predictive model the AUC was 0.88, specificity 0.73, sensitivity 0.94 and accuracy 0.89. The positive and negative predictive value of the model were 93.5% and 46.8%, respectively.
Limitations, reasons for caution: Employment of natural cycles may present as a limitation when examining the value of this study, as the cut-off values reported herein may be altered when stimulation is employed. Since internal validation may be confounded by the fact that this was a single center study, external validation is required.
Wider implications of the findings: The clinical end-point of this study reporting back to the practitioner, is the development of a predictive model identifying the optimal POR population for whom LuPOR practice is valuable. The high positive predictive value of this model may assist clinicians in identifying poor responders who will benefit from this approach.
Trial registration number: Not applicable

P-675 Endocrine disrupting toxic chemical affect the development of the male reproductive system but do not appear to affect the female reproductive system.

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Study question: To assess whether geographical variations in oestrogenic disruptor contamination affect both sexes equally: on spermatogenesis and oogenesis.
Summary answer: There is a clear relationship between oligozoospermia and genital malformations dependent on the accumulation of endocrine disruptors in the mother, but not on ovarian reserve.
What is known already: Our Centre has conducted several population-based studies on semen quality, and have analysed the semen assessment and the medical history of 1,239 volunteers aged 18 to 30 years.

The results showed a prevalence of oligozoospermia that was highest in Valencia (22.7%), Barcelona (22.7%) and the Basque Country (18.7%). These are the regions of Spain with the highest degree of industrialisation in the last 50 years. It was lowest in Galicia (8.5%) and Andalusia (13.7%), the regions with the least industry.
Study design, size, duration: This is a cross-sectional study with 10,443 healthy women aged between 20 and 45 years from eleven different communities in Spain. We determined AMH values for each age and in groups of 5 years. Linear regression analyses were used to calculate ovarian age
Participants/materials, setting, methods: All AMH values were assessed using an ELISA assay (AMH Gen II ELISA assay; Beckman Coulter, Brea, CA, USA). All samples were processed in the same central laboratory.
Main results and the role of chance: The mean age of the women was 36.6 years ± 4.3 years. Reference values for AMH, expressed as 25th, 50th, and 75th centiles by age and five year groups, were obtained. There were significant differences in AMH values between groups of women aged 30-<35 years, 35-<40 years, and 40-45 years. No significant differences were observed in AMH values in the first two age groups (20-<25 years, and 25-<30 years). The 50th centiles of mean AMH ranged from 3.45 to 0.72 ng/ml. AMH values were found to be significantly, and inversely, correlated to age ($r=0.35$; $p<0.001$). From the regression equation, the estimated yearly decrease in AMH was 0.2 ng/ml. The range of AMH values in Spanish women were higher than those reported for other European countries and the USA, and lower than those reported for China.

Statistically significant differences were found between the results from different geographical areas but no pattern was found to justify them. It was hypothesised that in the more industrialised areas AMH levels should be lower and that this would correspond to the higher prevalence of oligozoospermia in male volunteers, but this was not the case.
Limitations, reasons for caution: The results for males were assigned to the geographical area where their mother was raised in all cases. For females, it was not possible to do so in all autonomous communities.
Wider implications of the findings: These results suggest that oestrogenic disruptors stored in maternal fat interfere with the action of testosterone in the foetal testis inducing testicular dysgenesis syndrome. But it does not affect embryonic/fetal ovarian development, we propose that this is because higher oestrogen levels do not alter the process.
Trial registration number: Not applicable

P-676 Mild stimulation followed by embryo accumulation via vitrification appears to be beneficial for managing poor ovarian response: A retrospective cohort study including 610 patients

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Study question: Could embryo accumulation employing mild stimulation cycles prove beneficial for managing patients presenting with poor ovarian response (POR)?
Summary answer: Embryo accumulation may be an efficient POR management strategy, enabling a higher number and quality cohort of embryos, ultimately improving success results.
What is known already: It is widely accepted that POR constitutes a challenging condition. The limited oocyte yield associated with POR detrimentally impacts in vitro fertilization (IVF) success rates. Moreover, the documented heterogeneity among POR patients compromises our efforts to successfully address POR, despite the advances noted regarding stimulation protocols employed today. Considering the aforementioned, embryo accumulation following consecutive stimulation cycles has emerged as an alternative management strategy towards increasing the number of available embryos prior to embryo transfer (ET), mimicking normoresponding conditions. However, only few studies have been so far conducted and the need for further data is underlined.
Study design, size, duration: A single-center retrospective study was conducted in the Centre of Human Reproduction, Genesis-Athens Clinic from January 2015-December 2019. Only patients presenting with POR according to Bologna criteria were included. In total, 610 POR patients were considered eligible and were divided in three groups namely, mild stimulation-fresh ET (150 IUs of gonadotropins) (MILDF), mild stimulation employing embryo accumulation (MILDA), and natural cycle employing embryo accumulation (NATA). Respective comparisons on embryology and pregnancy data are provided.
Participants/materials, setting, methods: Resulting embryos from the MILDF, MILDA, and NATA groups were cultured up to the cleavage stage and categorized into three groups according to quality, namely top (grade 1), good (grade 2-3) and poor (grade 4-5) (Veeck, 1999). Top and good quality embryos were considered eligible for ET/vitrification. The banking scenario entailed accumulation of at least three embryos, including at least one top quality embryo. Embryo transfers included up to two cleavage stage embryos.
Main results and the role of chance: Comparing MILDF and MILDA groups, a higher number of available oocytes and embryos was observed in MILDA (2.36±1.15 vs 6.58±1.11; 1.72±1.02 vs 3.51±0.61, P-*value*<0.001). However, a mean number of 3.90±1.56 oocyte retrievals were required to conclude MILDA compared to MILDF which was concluded following a single oocyte retrieval (P-*value*<0.001). Cancellation-rate was significantly lower in the MILDA compared to MILDF group (0% vs 18.93%, P-*value* <0.001). A higher proportion of top quality embryos were transferred in the MILDA group (66.58% vs 43.67%, P-*value*<0.001). The MILDA group presented with higher positive-HCG (27.89% vs 23.30%, P-*value*=0.302), clinical-pregnancy (22.11% vs 17.96%, P-*value*=0.316) and live-birth rates (16.84% vs 14.08%, P-*value*=0.487). However, these differences were not significant. Comparing MILDA and NATA groups, the MILDA presented with a lower number of required oocyte retrievals and a higher number of oocytes per oocyte retrieval compared with NATA (3.90±1.56 vs 7.15±1.80; 1.95±0.74 vs 0.89±0.20, P-*value*<0.001). Moreover, the MILDA presented with a higher mean number of resulting embryos (5.20±0.78 vs 4.82±0.88, P-*value*<0.001). No difference was observed regarding the proportion of the resulting top quality embryos. The MILDA group presented with slightly higher clinical-pregnancy (22.11% vs 20.09%, P-*value*=0.628) and live-birth (16.84% vs 14.02%, P-value=0.490) rates, however these differences were not significant.
Limitations, reasons for caution: The retrospective nature of the study constitutes a major limitation. Considering that numerous confounders are inevitable when retrospective data is analyzed, authors employed strict eligibility criteria in an effort to reduce bias. Statistical analysis revealed a well-controlled population, considering that general patients' characteristics did not differ between the three groups.
Wider implications of the findings: Embryo accumulation may constitute an efficient management strategy for POR, as more embryos of better quality are available for ET compared to fresh-IVF-ET. Mild stimulation should be preferred for embryo accumulation instead of natural cycles, as less oocyte retrievals are required. Future studies should be conducted to verify these conclusions.
Trial registration number: Not Applicable

P-677 Endometrial thickness, endometrial preparation protocol and number of euploid embryos transferred, significantly impact the live birth in frozen embryo transfer cycles

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Study question: Is the live birth rate (LBR) in euploid frozen embryo transfer (FET) cycles affected by the endometrial thickness (EMT)?
Summary answer: A significantly higher LBR was observed in patients with an endometrial thickness of at least 7.5mm (46.24% vs. 54.63%)
What is known already: Parameters assessing the endometrium prior planning a FET include endometrial thickness, pattern and blood flow. The impact of the endometrial thickness on ART outcomes is controversial, with conflicting results published. A recent meta-analysis evaluated whether EMT could predict pregnancy outcomes and suggested that lower EMT was associated with lower incidence of clinical pregnancy rate (CPR), implantation rate (IR) and LBR. Due to heterogeneity of parameters evaluated between different publications, where embryos with unknown ploidy status were transferred, in conjunction with variability of stimulation protocols and the number of embryos transferred, the real effect of the EMT was difficult to infer.
Study design, size, duration: This was a two-center retrospective observational study including a total of 1522 euploid FET cycles between March 2017 and March 2020 at ART Fertility Clinics Muscat, Oman and Abu Dhabi, UAE.
Participants/materials, setting, methods: Trophectoderm biopsies were analyzed with Next Generation Sequencing (NGS). Vitrification/warming of blastocysts was performed using Cryotop method (Kitazato). EMT was measured by vaginal ultrasound prior initiating the progesterone administration (± 1 day) and LBR was recorded. Multivariate analysis was performed between LB outcomes and median EMT while controlling for confounding factors.
Main results and the role of chance: A total of 1522 FET cycles were analyzed: 975 single embryo transfer (SET) and 547 double embryo transfer (DET). The mean age of the patients was 33.38 years with a mean BMI of 27.1 kg/m2. FET were performed in EMT ranging from 3 to 15 mm and 50.52% resulted in a live birth. Though potentially all ranges of EMT were associated with LB, the median EMT in patients with LB was significantly higher than the median EMT of patients without LB (7.6mm vs. 7.4mm; $p < 0.001$).

The dataset was stratified into two groups based on the median EMT (7.5mm): < 7.5mm (n=744 cycles) and ≥ 7.5mm (n=778 cycles). A significantly higher live birth rate was observed in ≥ 7.5mm group (46.24% vs. 54.63%. p=0.0012).

In multivariate analysis, EMT, FET endometrial preparation protocol, and number of embryos transferred were the main parameters influencing the chance to achieve LB: OR 1.10 [1.01-1.19], p<0.015 for the EMT; OR 1.84 [1.47-2.30], p<0.0001 for Natural Cycle protocol and OR 1.55 [1.25-1.93], p<0.0001 for DET. Intercept 0.18 [0.07-0.44] p<0.0002. Female age did not reach significance: OR 1.02 [1.00-1.04], p=0.056.
Limitations, reasons for caution: Besides the retrospective nature of the study, the inter-observer variability in EMT assessment between different physicians is a limitation. The physician and embryologist performing the embryo transfer could not been standardized due to the multicenter design of the study.

Wider implications of the findings: The EMT in FET may influence the LBR and should be considered as an important factor for the success of embryo transfer cycles. Whether these results can be extrapolated to fresh embryo transfer and to blastocysts with unknown ploidy status, needs further investigation.
Trial registration number: not applicable

P-678 Increased luteinizing hormone in ovarian dysfunction attenuates follicle development and oocyte quality in human

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Study question: Can increased luteinizing hormone impair follicular development and oocyte quality in patients with ovarian dysfunction?
Summary answer: Increased luteinizing hormone attenuates follicular development and oocyte quality, resulting in arrest of follicle growth and empty follicles and low-quality embryos.
What is known already: Patients with ovarian dysfunction exhibit elevated gonadotropins and low estrogen levels reflecting their low ovarian reserve. For ovarian stimulation in these patients, natural or mild stimulation protocols are likely used, but we often experienced the arrest of follicle growth and empty follicles at oocyte retrieval. Animal studies demonstrated that chronic high LH exposure impaired the growth of antral follicles by suppressing the expression of FSHR in granulosa cells via a modulation of intraovarian regulators, including the LH-induced thecal factors. Study design, size, duration: Retrospective analysis was conducted in 72 patients with ovarian dysfunction who received ovarian stimulations followed by IVF-ET from April 2018 to March 2020 after obtaining written informed consents under an approval from the ethical committee of our hospital.
Participants/materials, setting, methods: The data of hormonal levels, transvaginal ultrasound during ovarian stimulation and clinical outcome of IVF were extracted from electric chart. For evaluation of embryo, high quality embryos referred to embryos having Veeck classification >grade 3 and >4 blastomeres. Statistical significance was determined using Dunnett or chi-square tests, with P <0.05 being statistically significant.
Main results and the role of chance: The median age of participants was 42 years of age (range 26-49) with low serum AMH levels (median 0.9 ng/ml, range 0-1.83). We analyzed 361 cycles of ovarian stimulation in total (median 4 cycles/patient, range 1-21). These stimulation cycles were classified into 3 groups; group A (n=230): normal LH level, group B (n=93): elevated LH level (> 10 mIU/ml) after ovarian stimulation and group C (n=33): elevated LH level from the initiation of ovarian stimulation. Among 361 cycles, the arrest of follicle growth was detected in 5 cycles (group A: 0%, group B: 60%, group C: 40%). The proportions of empty follicle in group A, B and C were 17.3±2.0%, 20.9±3.3%and 38.6±7.2%, respectively. The rate of empty follicle was significantly high in group C. Although there was no significant difference in the rates of oocyte degeneration and fertilization, the rate of high-quality embryos in group C was 0.8-fold lower than that of group A.
Limitations, reasons for caution: Due to limitation of participants, we could not determine the appropriate LH level for ovarian stimulation in patients with ovarian dysfunction based on receiver operatorating characteristic curve.
Wider implications of the findings: Normalization of LH levels for ovarian simulation in patients with ovarian dysfunction could improve follicle development and oocyte quality.
Trial registration number: not applicable

P-679 Magnetic resonance imaging (MRI), an alternative method to evaluate the ovarian reserve

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Study question: Is magnetic resonance imaging (MRI) a valid method to access antral follicles count (AFC) compared to two-dimensional (2D) transvaginal ultrasonography (USG) and Anti-Mullerian Hormone (AMH) to evaluate the ovarian reserve?
Summary answer: AFC has a high agreement between MRI and USG methods, as well as with AMH. MRI can be an alternative method to evaluate ovarian reserve.
What is known already: In fact, two methods are the most used today to assess ovarian reserve: USG and AMH. The USG is considered the gold standard method for AFC, and contributes to predict and tailor treatment strategies, such as in-vitro fertilization. The major limitations of USG are its user dependency and equipment. Also, there are limitations in displaying a global view of the pelvis and large ovarian lesions. Magnetic resonance imaging (MRI), with its excellent soft-tissue contrast resolution and characteristics, is a useful non-invasive alternative modality to USG. Just one study evaluated MRI and revealed more small size antral follicles compared to 3D-USG.
Study design, size, duration: A prospective cross-sectional observational study was performed in an assisted reproduction techniques (ART) post-graduation program setting from an assisted reproductive center in Sao Paulo, Brazil, which ran throughout 2019-2020, with a total number of 59 patients that were in fertility treatment and needed to undergo to a MRI procedure to evaluate uterine or pelvic diseases as, Myoma, Adenomyosis, endometriosis, and adnexal cysts.
Participants/materials, setting, methods: Patients were evaluated to access the AFC by the MRI method and 2D transvaginal USG, and Anti-Mullerian Hormone (AMH) concentration to evaluate the ovarian reserve. Comparison between methods was done through Wilcoxon signed ranks test and Bland-Altman analysis. Ovarian reserve was classified as follows: very low (<4 follicles/AMH<0.5); low (5-7 follicles/AMH=0.5-1.1); normal (8-15 follicles/AMH=1.1-3.5); normal-high (>15 follicles/AMH>3.5). Weighted Cohen's kappa was used to verify agreement between MRI, USG and AMH classifications of ovarian reserve.
Main results and the role of chance: Average AFC for USG were 5,55±4.01 for left ovary and 5.55±3.8 for right ovary. Average follicle count for both ovaries was 10±7.07. Regarding MRI, mean counts were 6.44±4.81 for left ovary, 5.65±3.85 for right ovary, and a 11.89±7.89 follicle sum mean count. Average concentration of AMH was 1.79±1.44. The Wilcoxon test and Bland-Altman analyses found differences and systematic biases for comparison between USG and MRI for both ovaries (-2.58; limits of agreement=-14.56 to 9.40, Wilcoxon p=0,008) and for the right ovary (-1.48; limits of agreement=-8.32 to 5.35, Wilcoxon p=0,031). There was no difference between methods for the left ovary. Weighted Cohen Kappa coefficients showed substantial agreement between ovarian reserve classifications based on AMH levels, USG, and MRI. The conducted paired comparisons were USG with MRI (k=0.676), AMH with MRI (k=0.760) and USG (k=0.609).
Limitations, reasons for caution: The systematic biases found when comparing USG to MRI methods may suggest a consistent detection of more follicles with MRI procedures in comparison to the USG method. This bias found warrants caution as it must be confirmed, in future studies.
Wider implications of the findings: The MRI method reveals similar ovarian reserve to USG when used the same classification, and a higher agreement to AMH. This suggests that MRI is a reliable method of quantifying antral follicles and can also be adopted when the patient will need to evaluate pelvic pathologies.
Trial registration number: not applicable

P-680 Thyroid function in euthyroid women during controlled ovarian stimulation (COH): does the TSH fluctuations have an impact on IVF outcomes?

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Study question: TSH blood levels play a role in terms of ovarian stimulation and pregnancy? Do we need to treat patients with TSH out of range?
Summary answer: Euthyroid patients with negative autoantibodies during COS should not be treated even if basal TSH is higher than 2.5 U/L
What is known already: Abnormal thyroid function is associated with adverse pregnancy outcomes, being essential during embryo implantation and early pregnancy. TSH receptors can be found in endometrial and ovarian tissues and during controlled ovarian stimulation TSH levels suffer modifications because of hyperestrogenemia. Subclinical hypothyroidism is defined as a TSH level greater than 4.5 mIU/L with normal FT4 levels. It is controversial whether or not to use first-trimester pregnancy and infertility thresholds for upper limit of 2.5 mIU/L TSH. However, neither American Thyroid Association nor the American Society Reproductive Medicine recommendations have clearly defined how infertile patients need to be treated.
Study design, size, duration: Between April 2016 and December 2019, we performed a retrospective observational study at the University Hospital of Padua, including patients who underwent IVF/ICSI treatments. We included patients with idiopathic or tubal infertility or with poor ovarian reserve, in presence of basal TSH≤ 4,5 U/L and negative anti-TPO Ab and anti-Tg Ab. Exclusion criteria were severe male factor, TSH<0,2 or >4,5 U/L, BMI higher than 30, oncologic patients, uterine disease.
Participants/materials, setting, methods: We enrolled a total of 389 patients. We checked TSH blood levels on the day before starting stimulation (T0). We divided our patients according to TSH level < 2,5U/L (group A) or ≥2,5U/L (group B). We then checked TSH on the day of hCG trigger (ThCG). Delta TSH (ThCG-T0) was calculated and correlated to endometrial thickness, number of occytes retrieved, embryos obtained and frozen, Clinical Pregnancy Rate (CPR) and Live Birth Rate (LBR).
Main results and the role of chance: Group A (251) and group B (138) were homogeneous for age, body mass index, AMH levels, antral follicular count. Short ovarian stimulation cycle with GnRH antagonist and long cycle with GnRH agonist proportions were similar in both groups. Also, FSH recombinant and hMG gonadotropin use and total amount were similar for the two groups. No statistically significant difference was found between the groups in terms of endometrial thickness, follicles greater than 14 mm as medium diameter, number of oocytes retrieved, number of mature oocytes (MII), embryos obtained, number of embryos cryopreserved, CPR and LBR. However, when considering the Delta TSH, we found possible correlations in group A. In particular, the number of oocytes retrieved was higher in Delta TSH positive (3.4±2.2) rather than in Delta negative women (2.6±1.7) with a p value of 0.002. Moreover, mature oocytes (MII) were retrieved more frequently in Delta TSH positive (5.7±3.8) rather than in Delta negative women (4.5±3) with a p value of 0.008. Group B Delta TSH did not show any possible statistically significant correlations.
Limitations, reasons for caution: This is a retrospective study and results must be confirmed on a well-designed randomized controlled study. Moreover, since we use strict eligibility criteria, we enrolled few patients. Correlations must be considered with caution since the role of TSH is under study, especially when considering LBR outcome.
Wider implications of the findings: Our results are congruent with previous studies. In particular, we suggest not to treat patients with TSH levels on range (between 0.2mIU/L and 4.5 mIU/L). TSH increase during COS in euthyroid patients could be interpreted as a positive physiological response and it is associate with better COS outcomes.
Trial registration number: N/A

P-681 Will the hCG trigger dose used for final oocyte maturation in IVF impact endogenous progesterone during the luteal phase? - A randomized controlled trial

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Study question: Is there an association between the hCG dose used for ovulation trigger and the endogenous progesterone production during the luteal phase?
Summary answer: Increased hCG dosing significantly increased the endogenous progesterone level during the luteal phase.
What is known already: During the luteal phase of an IVF treatment, the endogenous progesterone (P4) production is negatively impacted due to reduced circulating endogenous LH, caused by negative feed-back of elevated steroids; thus, luteal phase support (LPS) with exogenous P4 remains mandatory in IVF. Apart from inducing final oocyte maturation, the gold standard HCG trigger also functions as an early LPS, boosting P4 production by the corpora lutea (CL). P4 plays a pivotal role for embryo implantation and pregnancy, and an optimal P4 level around peri-implantation seems to be essential for the reproductive outcomes of fresh and frozen/thaw embryo transfer cycles.
Study design, size, duration: A randomized controlled 4-arm study, including a total of 127 IVF patients, enrolled from January 2015 until September 2019 at the Fertility Clinic, Odense University Hospital, Denmark.

Participants/materials, setting, methods: IVF patients with ≤ 11 follicles ≥ 12 mm were randomized to four groups. Groups 1-3 were triggered with: 5.000 IU, 6.500 IU or 10.000 IU, hCG, respectively, receiving a LPS consisting of 17- -hydroxy-progesterone (17 OH P4) to distinguish the endogenous P4 from the exogenous supplementation. Group 4 (control) was randomized to a 6.500 IU hCG trigger and standard LPS. A total of eight blood samples were drawn during the early luteal phase.
Main results and the role of chance: A total of 94 patients completed the study: 21, 22, 25 and 26 patients in each group, respectively. Baseline characteristics were similar, except for the endogenous LH level and cycle lengths. There were no significant differences between groups regarding ovarian stimulation, number of oocytes and embryos. The median number of follicles ≥ 12mm on the day of trigger was 8.5, resulting in 6.6 oocytes being retrieved. Significant differences in P4 levels were seen at OPU+8 (p<0.001), OPU+10 (p<0.001) and OPU+14 (p<0.001), with positive correlations between P4 level and hCG dose. Groups compared individually showed significant difference in P4 between low and high trigger dose at OPU+4 group 1 and 3 (p= 0.037) and OPU+8 group 1 and 3 (p=0.007) and between all the three groups around implantation at OPU+6 group 1 and 2 (p=0.011), group 2 and 3 (p=0.042) and group 1 and 3 (p< 0.001). Higher P4 levels around implantation were related to follicle count and to pregnancy. After logistic regression analyses there were still significant individual differences between the groups.
Limitations, reasons for caution: Although patients were randomized and strict inclusion and exclusion criteria were used, the RCT was un-blinded, including a relatively small number of patients. Moreover, for dosing purposes urinary hCG as well as recombinant hCG was used and pharmacokinetics differ. Finally, the P4 level could be influenced by circadian fluctuations.
Wider implications of the findings: This is the first study to explore dose-responses in circulating P4 after hCG trigger in IVF patients. Increasing the hCG trigger dose increased the endogenous P4 around peri-implantation. Personalizing the hCG trigger dose could be a key point to secure the most optimal P4 mid-luteal phase P4 level.
Trial registration number: Eudract 2013-003304-39

P-682 Serum progesterone level as prognostic factor in frozen-thawed embryo transfer cycles: effect of selected threshold on gestational results. Systematic review, stratified meta-analysis and meta-regression.

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Study question: Is there an optimum progesterone threshold level below which gestational results are significantly worse in frozen embryo transfer cycles (FET) with hormone replacement therapy (HRT)?
Summary answer: Low serum progesterone during luteal phase of HRT-FET cycles impairs substantially its gestational outcomes, regardless of threshold level, origin of oocytes and euploidy of embryos.
What is known already: HRT for endometrial preparation in FET or oocyte donation cycles is widely used. Oestrogen doses are usually patient-tailored varying upon endometrial thickness, whereas the optimal level of progesterone exposure has not been defined. Various studies have found a negative association between serum progesterone levels measured during luteal phase and FET results in terms of pregnancy and miscarriage rates. Most likely there is an optimal level below which results are worse but a standard threshold level is yet to be established, as in almost every study a different threshold has been found.
Study design, size, duration: Systematic review and stratified meta-analysis with meta-regression following PRISMA guidelines. An electronic search of MEDLINE, EMBASE, Web of Science, Cochrane Gynaecology and Fertility Specialised Register of Controlled Trials and ClinicalTrials.gov was conducted from inception to January 2021. The aim was to identify prospective or retrospective cohort studies measuring serum progesterone levels around frozen embryo transfer date in HRT cycles. A combination of the following key search terms was used: “progesterone”, “serum”, “frozen embryo”, “transfer”, “frozen-thawed”.
Participants/materials, setting, methods: Studies analyzing association of luteal serum progesterone with FET-HRT outcomes were included. Risk of bias within studies was assessed using the Newcastle-Ottawa Scale (NOS). Clinical/ongoing pregnancy and miscarriage rates (C/OPR,MR) were considered as primary and secondary outcomes respectively. Odds Ratios with 95% Confidence Interval (OR,95%CI) were calculated applying a random effects model meta-analysis. Heterogeneity was assessed using the I2 statistic. A meta-regression was conducted to examine the association of the effect with the threshold level.
Main results and the role of chance: The systematic search retrieved 792 studies, 494 after duplicates removal of which 343 were screened and 51 assessed for eligibility. 12 studies, reporting 14 threshold levels, were included in the meta-analysis involving 5009 HRT-FET cycles. Two of them were prospective cohort studies while the rest were retrospective. 10 of them have been published in peer review journals and two were conference abstracts. Quality of studies assessed with NOS varied between 5 and 9. The progesterone threshold ranged from 5.0 to 21.94 ng/ml. Low progesterone levels were associated with less C/OPR (OR: 0.52; 95% CI: 0.40 to 0.66; 11 studies, 5009 cycles). Low progesterone was also associated with high MR (OR: 2.01; 95% CI: 1.57 to 2.58; 9 studies, 2560 pregnancies). These effects showed remarkable consistency in specific sub-analyses considering separately studies with progesterone thresholds up to or above 10 mg/mL, and studies carried out in cycles using oocyte donation, autologous oocytes and embryo aneuploidies screening. Meta-regression did not identify significant association between size effect and progesterone threshold, regarding neither C/OPR (regression coefficient: 0.02; CI 95%: -0.02 to 0.06; p: 0.28) nor MR (regression coefficient: 0.11; CI 95%: -0.13 to 0.36; p: 0.32).
Limitations, reasons for caution: High degree of clinical and statistical heterogeneity was found due to different routes and doses of progesterone administration, date of progesterone analyses and variety of thresholds as well as high diversity of embryo origin. Despite sensibility analysis by embryo origin any of these sources of heterogeneity can preclude the results.
Wider implications of the findings: Despite low progesterone levels are significantly associated to lower gestational results, and a threshold of 10 ng/ml constitutes the median value of our distribution, high quality prospective studies are needed to validate the prognostic value of progesterone levels and to establish an standardised threshold level for clinical application.
Trial registration number: not required

P-683 Correlation of vitamin D deficiency with anti-Mullerian hormone (AMH) among infertile women in comparison to fertile women in a tertiary health facility in North-Western Nigeria

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Study question: is there correlation between Vitamin D Deficiency and AMH levels in infertile and fertile women?
Summary answer: there is no significant correlation between Vitamin D deficiency and AMH levels in both infertile and fertile women
What is known already: Vitamin D deficiency and insufficiency is a global health problem affecting over a billion people with higher prevalence among reproductive-age women, and blacks. Vitamin D is well known to play significant role in calcium-phosphate homeostasis and bone metabolism, however, recent studies have demonstrated diverse expression of vitamin D receptors in reproductive organs. This suggest the probable role of vitamin D in reproductive physiology and fertility. The pathogenesis of vitamin D in infertility is poorly understood, but thought to involve hypothalamo-pituitary axis, ovarian folliculogenesis and uterine implantation. Most studies are done in Assisted Reproduction Technology and in developed countries
Study design, size, duration: A case-control study that involved 128 consecutively consenting women within the reproductive age group; 64 infertile women as the cases and 64 age and body mass index (BMI) matched fertile women as the controls. The study was conducted over a period of six (6) months
Participants/materials, setting, methods: The study was conducted in Obstetrics and Gynaecology and Chemical pathology departments of Ahmadu Bello University Teaching Hospital Zaria, a tertiary hospital in North-Western Nigeria. It involved all cases of female-factor infertility as cases, while the controls were fertile women from 6 weeks postpartum to 1 year. Venous blood samples were assayed for serum 25(-hydroxy) vitamin D and AMH levels using Enzyme-Linked Immunosorbent Assay (ELISA) and data analysed with level of significance set as <0.05
Main results and the role of chance: The mean ± standard deviation (SD) of serum Vitamin D levels in the infertile women and fertile women were 17.01 ± 7.61ng/ml and 11.34 ± 6.12ng/ml respectively, significantly higher in the infertile women (p-value <0.000). The prevalence of Vitamin D deficiency (<20ng/ml) was found to be significantly higher in the fertile women compared to infertile women (89.1% versus 68.8%; p-value 0.007). Vitamin D levels were found to be positively correlated with age (r 0.374; p-value 0.002) and parity (r 0.338; p-value 0.006). There was no association between vitamin D with type of, and causes of infertility. Vitamin D deficient women were found to be 6.5 times less likely to be infertile than non-deficient women (aOR 95% confidence interval 1.96-21.55; p-value 0.002). There was no significant correlation between vitamin D and AMH levels in vitamin D deficient women of both study groups (rs 0.180; p-value 0.242 and rs 0.088; p-value 0.521). Interestingly, there was significant relationship between AMH levels and causes of infertility (p-value 0.001), with higher levels of AMH found in infertile women with polycystic ovarian syndrome
Limitations, reasons for caution: There is no consensus on the cut-off values for vitamin D levels as it relates to fertility, and no reference values for vitamin D deficiency and AMH levels in study area. The sample size was limited by cost, and the study was conducted in a single study area
Wider implications of the findings: The vitamin D levels in women with infertility was low but yet not significantly correlated with AMH. Overall, prevalence of vitamin D deficiency among reproductive-age women was found high. There is need for life-style and dietary modifications. Further researches are needed to ascertain the effect of vitamin D on fertility.
Trial registration number: not applicable

P-684 Impact of GnRH antagonist pretreatment on oocyte yield after ovarian stimulation: a retrospective analysis

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Study question: Does a 3-day pretreatment course with a GnRH antagonist in the early follicular phase increase the number of oocytes in a GnRH antagonist stimulation protocol?
Summary answer: The administration of 3 days of GnRH antagonist before starting ovarian stimulation in a GnRH antagonist protocol increases the number of COCs (Cumulus-Oocyte-Complexes).
What is known already: The GnRH antagonist protocol is characterized by higher gonadotropin and E2 serum levels at the start of ovarian stimulation (OS), compared with a long pituitary down regulation protocol. The unsuppressed FSH level at the start of a GnRH antagonist cycle allows the initial growth of follicles before addition of exogenous FSH, which may result in asynchrony of the follicular cohort. Menstrual administration of a GnRH antagonist can inhibit follicle growth and improve homogeneity of recruitable follicles. Previous studies showed a trend toward higher numbers of COCs and improved maturation and fertilization rates of retrieved oocytes.
Study design, size, duration: Retrospective single center crossover study, including consecutive women enrolled in an IVF program in a university hospital from January 2011 to December 2020. All women underwent one standard GnRH antagonist stimulation cycle ("standard cycle") and one GnRH antagonist stimulation cycle preceded by early administration of GnRH antagonist for 3 days ("pretreatment cycle"). Women with basal progesterone levels >1.5ng/ml, and women undergoing oocyte freezing, oocyte donation or PGT were excluded. In total, 427 patients were included.
Participants/materials, setting, methods: Women were included when the pretreatment cycle occurred within a time interval of <12 months following the start of stimulation in the standard cycle. The primary outcome was the total number of COCs.
Main results and the role of chance: The average female age was 35.1 ± 4.7 years. Indications for fertility treatment included unexplained infertility (34.3%), male-factor infertility (33.3%), age (16.9%), PCOS (8.2%) and endometriosis (2.6%). All cycles were divided into two groups: group 1 (standard, 427 cycles) and group 2 (pretreatment, 427 cycles). The mean duration of stimulation was similar in both groups (10.3 vs 10.3 days, p=0.2). The starting dose of gonadotropin (196.8 vs 234.9IU, p<0.001) and total amount of gonadotropin used (2000.7 vs 2415.2IU, p<0.001) were higher in group 2. The total number of obtained COCs (6.2 vs 8.8 p<0.001) and the number of mature oocytes (4.2 vs 6.4 p<0.001) were significantly higher in group 2. The Generalized estimating equation (GEE) multivariate regression analysis showed that the pretreatment strategy had a significant positive effect on the number of COCs (coefficient 2.8, p value <0.001 after adjusting for the confounders age, indication of infertility, stimulation dose, type and total amount of gonatropins used).
Limitations, reasons for caution: Despite the large dataset, the presence of biases related to the retrospective study design cannot be excluded. Besides, the impact of GnRH pretreatment on pregnancy rate cannot be assessed because of the crossover design.
Wider implications of the findings: A 3-day course of GnRH antagonist pretreatment increases the number of COCs obtained after OS. Furthermore, since the initiation of OS in a GnRH antagonist protocol relies on the occurrence of spontaneous menses, addition of three days of GnRH antagonist pretreatment may enhance scheduling flexibility without reducing efficacy.
Trial registration number: not applicable

P-685 Luteinization after final oocyte maturation induction is not compromised in women that receive double dose of Gonadotrophin-releasing hormone antagonists during controlled ovarian hyperstimulation

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Study question: Does the use of double dose of GnRH antagonists during COH in women with risk of premature LH surge alter luteinization after final oocyte maturation induction?
Summary answer: The use of double dose of GnRH antagonist in women with risk of premature luteinizing hormone surge dosent affect luteinization after final oocyte maturation induction.
What is known already: GnRH antagonists are used to prevent a premature LH surge during controlled ovarian hyperstimulation. The antagonists directly inhibit gonadotrophin release within several hours through competitive binding to pituitary GnRH receptors, producing a rapid suppression of LH and FSH, with no initial flare effect. In women with diminished ovarian reserve (DOR) it is not uncommon that premature luteinization cannot be completely prevented using a daily dose GnRH antagonist. To date, no study has evaluated the effects of using a daily double dose of GnRH antagonists to prevent a premature LH surge and its effect on luteinization after final oocyte maturation induction.
Study design, size, duration: This monocentric retrospective analysis evaluated the effect on luteinization after final oocyte maturation induction in twenty women during COH who received a daily double dose of GnRH antagonists (Cetrotide 0.25 mg/mL, Merck) from January 2020 to December 2020.
Participants/materials, setting, methods: Women with severe DOR and history of premature luteinization during COH received a double dose of GnRH antagonist when the leading follicle reached 12-14 mm (am and pm). When two follicles reached ≥18 mm in diameter, final oocyte maturation was induced with dual trigger using Leuprolide acetate and hCG. Progesterone, estradiol, bHCG, and LH levels were measured the day after final oocyte maturation induction to assure adequate luteinization.
Main results and the role of chance: In total twenty women were included in the analysis. Mean age 36.8± 4.2, AMH 0.65± 0.32 ng/ml, baseline antral follicle count 4± 2.3, serum hormone levels the day of ovulation induction trigger: progesterone 0.89± 0.34 ng/ml, LH 1.6± 2.1 ng/ml, estradiol 1235 ± 1420 pg/ml. Post-surge serum hormone levels average reached adequate levels: estradiol 1645 ± 1116 pg/ml, progesterone 20.4 ±2.2 ng/ml, LH 62.66± 10.5 IU/ml and, bHCG 247±115 IU/ml. A total of 76 oocytes were retrieved (3.8± 0.8 oocytes per patient), 63.1% (48/76) MII, 22% (17/76) MI, 14% (11/76) GV.
Limitations, reasons for caution: The retrospective nature of the study, small sample size, and potential variability in the study center's laboratory protocol(s) compared to other reproductive treatment centers may limit the external validity of our findings.
Wider implications of the findings: The daily use of double dose of GnRH antagonists during COH offers the possibility of preventing a premature LH surge in women with DOR with high risk of early ovulation, without compromising luteinization after final oocyte maturation induction.
Trial registration number: NA

P-686 safety & efficacy of the combination therapy of inositols, antioxidants and vitamins in polycystic ovarian syndrome (PCOS): A multicentric, retrospective observational study (Trazer study)

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Study question: Does combinations therapy of insulin sensitizing agents, antioxidants and vitamins are safe and efficacious in PCOS patients.

Summary answer: Combination therapy of inositols, antioxidants and vitamins is safe and effective non-hormonal treatment option to manage PCOS.
What is known already: Monotherapy of insulin sensitising agents, antioxidants and vitamins is beneficial in the treatment of PCOS. Nutritional supplement containing inositols, N-acetylcysteine (NAC), lycopene, chromium picolinate, vitamin D3, biotin and folic acid treatment resulted in a significant improvement in menstrual cyclicity, acne and hirsutism. But there is no evidence pertaining to the hormonal parameters and ovarian morphology. Therefore, the present investigation was planned to evaluate the effects of combination therapy of inositols, antioxidants and vitamins on sign and symptoms, metabolic and hormonal parameters in women with PCOS.
Study design, size, duration: Multicentric, retrospective, observational cohort study was planned for the first time at thirty-six fertility clinics in different states of India from April 2019 to November 2020. Both lean and obese patients (16-39 years; n=180) with confirmed diagnosis of PCOS as per the Rotterdam/ESHRE criteria were included in study.
Participants/materials, setting, methods: Patients were received a combination therapy of insulin sensitizers, antioxidants and vitamins in a marketed formulation (Trazer F ForteTM) twice daily as a tablet for 3 months and thereafter ovulation induction was done using letrozole (2.5-5 mg). Primary outcomes were improvements in signs of PCOS (menstrual cyclicity or ovulation restoration, acne and hirsutism), body weight, body mass index, waist circumference, ovarian cysts, pregnancy rate and hormonal balance. Secondary outcome was the evaluation of side effects.
Main results and the role of chance: Combination therapy of Trazer F ForteTM containing insulin sensitising agents (inositols, NAC and chromium), antioxidants (NAC and lycopene), and vitamins (vitamin D, biotin and folic acid) showed significant improvement in menstrual cyclicity by 54.3% and 88.2% in obese PCOS cases, and 48.7% and 79.5% in lean PCOS cases after 3- and 6-month of intervention respectively. Significant improvement was observed in acne, hirsutism and ovarian cysts post-intervention in both obese and lean PCOS women. After successful completion of the treatment, significant corrections were observed in metabolic (fasting glucose, fasting insulin and HOMA-IR) and hormonal profile (free testosterone, LH:FSH ratio, AMH and progesterone) in obese as well as lean PCOS cases. The clinical pregnancy rate was 16.2% and 34.1% in obese women, and 9.9% and 22.1% in lean cases after 3- and 6-month of intervention respectively.
Limitations, reasons for caution: Prevalent of PCOS is different in different population in India with diverse ethnic background. Hence, community-based intervention studies on larger population are needed to assess the efficacy and safety of such combinations amongst different age groups of women with PCOS.
Wider implications of the findings: Since, PCOS is a multifactorial disorder, combined use of inositols, antioxidants and vitamins can be used as a promising and clinically relevant non-hormonal treatment option for the management of PCOS.
Trial registration number: NA

P-687 Impact of medroxyprogesterone acetate (MPA) as pituitary suppression on oocyte quality and clinical outcomes in egg donation recipients

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Study question: To compare the impact on oocyte quality and reproductive outcomes in patients who received oocytes from donors stimulated with MPA versus GnRH antagonist protocol.
Summary answer: Compared to GnRH antagonist, MPA does not exert a major effect on oocyte quality and yields similar reproductive outcomes in egg donation recipients.
What is known already: Conventional ovarian stimulation (OS) protocols have classically used GnRH analogues, both agonists and antagonists, to avoid premature follicular luteinization. The oral administration of MPA or micronized progesterone during the follicular phase of OS has emerged as an attractive alternative to conventional protocols in the prevention of early luteinization. Compared to progesterone, MPA is characterized by a moderate-strong progestanic action, lower androgenic properties and does not interfere with the measurement of endogenous progesterone. In our group, administration of MPA during the follicular phase of OS has been included in the routine clinical practice of our donor program since late 2019.
Study design, size, duration: Multicentre, retrospective, observational, cohort study carried out in eleven private university-affiliated IVF centers. The present study included a total of 14,282 fresh ovum donation cycles performed from October 2017 to March 2020. Oocyte donors were recruited and stimulated under either MPA (n=4,665) or GnRHa (n=9,617) to suppress the pituitary during the follicular phase of OS, and GnRH agonist was administered to trigger final oocyte maturation in all the participants.
Participants/materials, setting, methods: Recipients were divided according to the protocol used for premature luteinization prevention during the follicular phase of the ovum donation matched-cycle: Group 1, recipients who received oocytes from donors treated with 10 mg/day of MPA (ProgeveraÒ); Group 2, recipients who received oocytes from GnRH antagonist (FyremadelÒ) down-regulated donor cycles. All the procedures were approved by an Institutional Review Board (1910-VLC-091-JG) and complied with Spanish law on assisted reproductive technologies (14/2006).
Main results and the role of chance: Regarding donor´s baseline characteristics, age and antral follicle count were significantly different between groups, but not clinical differences. The length of ovarian stimulation was similar in both groups (10.7 days [95% Confidence Interval (CI) 10.5-10-8] vs 10.5 days [95% CI 10.0-11.00]). Despite slightly higher mean total dose of FSH administered in Group 1 compared to Group 2 (1.841 IU [95% CI 1.813-1.868] vs 1.739 IU [95% CI 1.723-1.754]), there were no differences in the total dose of hMG administered between both groups (967 IU [95% CI 901-1.034] vs 971 IU [95% CI 944-998]). With regard to IVF data, both the number of retrieved oocytes (22.9 [95% CI 22.4-23.4] vs 24.1 [95% CI 23.8-24.3]), and mature oocytes (18.7 [95% CI 18.3-19.1] vs 19.3 [95% CI 19.1-19.6]), were slightly lower in Group 1 compared to Group 2, whereas fertilization rate was significantly higher in Group 1 compared to Group 2 (82.1% [95% CI 81.7-82.6] vs 80.8% [95% CI 80.6-81.2]),. Regarding the clinical outcomes, no differences were observed in either implantation rate (58.7% [95% CI 56.7-60.7] vs 59.3% [95% CI 57.3-61.3]) or clinical pregnancy rate (59.5% vs 59.8%, P=0.04) between both groups.
Limitations, reasons for caution: As a consequence of being a retrospective study, only association, and not causation, can be inferred from the results. A further limitation is that donors are healthy young women and do not perfectly match other populations, as infertile patients who may be older, low or high responders to OS.
Wider implications of the findings: MPA emerges as an effective oral alternative to GnRH analogues for preventing premature luteinizing hormone surges in donors undergoing OS in ovum donation program. Compared with GnRH antagonists, MPA has advantages of being an oral administration route and providing easy access, yielding similar clinical results.
Trial registration number: 1910-VLC-091-JG

P-688 Assessment of ovarian vascularity by three-dimensional vaginal power Doppler on day two of menstrual cycle to predict the number of mature eggs collected

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Study question: Could ovarian vascularity indices, measured by 3-dimensional (3D) vaginal power Doppler, predict the number of mature oocytes collected after controlled ovarian stimulation?
Summary answer: Ovarian vascularity index (VI) may be an indicator of poor (<three mature eggs collected) and high (>ten mature eggs collected) ovarian responses to gonadotropins.
What is known already: Poor and/or hyper ovarian responses to gonadotropins may be related to cycle cancellation during controlled ovarian stimulation (COS). In this context, gonadotropin dose is often individualized using patient features that predict ovarian response (such as age, antral follicular count (AFC) and anti-Müllerian hormone (AMH)). In parallel, ovarian vascularity color doppler is a valuable evaluation method to predict the ovarian hyperstimulation syndrome and the growth/maturity of Graafian follicles. The aim of the present study is

to estimate the utility of 3-dimensional vaginal power Doppler and ovarian vascular flow indices in the prediction of the number of mature occytes collected after COS.

Study design, size, duration: A prospective study was conducted on 200 couples undergoing intracytoplasmic sperm injection cycle at Al Hadi Laboratory and Medical center, Beirut, Lebanon. It was performed between January 2020 and July 2020. Couples were categorized into poor responders group (3 or less metaphase II (MII) eggs collected) (n=43), high responders group (10 or more MII eggs collected) group (n= 66), and normal responders group (more than 3 and less than 10 MII eggs collected) (n=66).

Participants/materials, setting, methods: On the second day of the menstrual cycle, ovarian volume and vascularity parameters (vascularity index (VI), flow index (FI), and vascularity flow index (VFI)) were measured using the 3D power Doppler and the Virtual Organ Computer-Aided Analysis. On the same day, the antral follicle count was evaluated and a blood sample for AMH testing was collected. Women included in the study have undergone COS using GnRH antagonist protocol.

Main results and the role of chance: Receiver operator characteristics (ROC) curve model was used to predict the number of mature eggs collected. 7 parameters were used to predict poor and high ovarian responses (Age, AMH, AFC, ovarian volume, VI, FI and VFI). Ovarian VI significantly predicted poor ovarian response to gonadotropins (p=0.033 and area under the curve (AUC)=0.668). Subsequently, the cut off value was 0.0025 with 84% sensitivity and 83.3% specificity. In parallel, ovarian VI significantly predicted high ovarian response to gonadotropins (p=0.036 and AUC (0.778)) with a cut off value 0.0375 and with 77.8% sensitivity and 78.3% specificity. Furthermore, VFI significantly predicted high ovarian response to gonadotropins (p=0.045; AUC=0.677).

Limitations, reasons for caution: It will be necessary to perform a prospective analysis on a broad sample size to validate these findings. In addition, it will be interesting to assess the impact of ovarian vascularity on pregnancy outcomes.

Wider implications of the findings: Assessing ovarian vascularity prior to ovarian stimulation can help reduce the rate of cycle cancellation. In addition, more studies are welcomed in the field to unravel the mechanisms behind altered ovarian vascularity and to test the possibility of restoring normal ovarian physiology.

Trial registration number: Not applicable

P-689 IL-6/IL-10 ratio as predictor of poor ovarian response in women undergoing in-vitro fertilization

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Study question: Are serum cytokines levels associated with ovarian response in IVF cycles?

Summary answer: The IL-6/IL-10 ratio is higher in patients with low ovarian response.

What is known already: Previous studies reported differences in the levels of IL-2, Il-6, IL-8, IL-10 and VEGF in follicular fluid between young patients with low ovarian response and normoresponder women. In addition, it is known that IL-6 plays an important role as a mediator of fever and acute phase reaction and IL-10 is the cytokine with the greatest anti-inflammatory power. Although there seems to be some evidence about the possible effect of the immune system on ovarian function and implantation, the role it plays in ART remains unknown. Our aim was to investigate the effect of cytokines in ovarian reserve and response.

Study design, size, duration: One hundred and fifty-two patients were included in a retrospective study between February 2016 and December 2020. Serum cytokines IL-2, IL-4, IL- 6, IL-8, IL-10, VEGF, IFNγ, TNFα, IL-1α, IL-1β, MCP-1 and EGF were measured previously to the ovarian stimulation cycle. Patients with altered karyotype, mutation or premutation in the FMR1 gene or endometriosis or with any other factor that could alter the ovarian reserve or response were excluded from the study.

Participants/materials, setting, methods: To measure the levels of the different cytokines, a sandwich immunoassay with specific antibodies for the cytokines IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFNγ, TNFα, IL -1α, IL-1β, MCP-1 and EGF were used. The statistical analysis was performed with R Statistical Software, version 4.0.3 and the Software Statistical Product and Service Solutions, version 20.0 (SPSS, Chicago, IL, EE.UU.).

Main results and the role of chance: We found that the ratio between IL-6 and IL-10 cytokines is higher in those patients in whom four or fewer oocytes have been recovered after ovarian puncture (2.15 versus 1.55; p = 0.035; Mann-Whitney test). If we establish 0.9 as a cut-off point for the IL-6 / IL-10 ratio, we observed that above this value the risk of having a low response to ovarian stimulation is more than 3 times greater than below this value (22.9% versus 6.0%; p = 0.007; Fischer exact test). There were no statistically significant differences between both groups in terms of age (p = 0.136), dose of gonadotropin administered (p = 0.415) and duration of ovarian stimulation (p = 0.706).

In addition, performing hierarchical cluster analysis with the analyzed cytokines and the associated variables to ovarian reserve and response, we observed that the antral follicle count, the total oocytes recovered and the MII recovered are grouped in the same cluster as the cytokines IL-2, IL-4, IL-6, IL-10, IL-1α, IL-1B, IFNγ y TNFα. We determined the number of clusters based on the tree diagram and k-means method.

Limitations, reasons for caution: The retrospective study design and the sample size could be a limitation. The study was performed in patients with suspected implantation failure.

Wider implications of the findings: The ratio between IL-6 and IL-10 could be used as a potential biomarker to predict the ovarian response and provide real expectations regarding the success of IVF cycle. The action of IL-6 could be reduced by blocking its receptor using humanized monoclonal antibodies as Tocilizumab.

Trial registration number: not applicable

P-690 Clinical predictors of a high oocyte maturation rate in IVF treatment cycles

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Study question: Which clinical parameters predict a high oocyte maturation rate in patients undergoing IVF treatment?

Summary answer: Time between oocyte collection and insemination demonstrated significant association with oocyte maturation and represents a parameter that could be optimised in IVF cycles.

What is known already: Oocyte maturation is an important factor determining IVF outcomes and can be a rate-limiting step for patients undergoing treatment. A number of clinical and laboratory variables may affect this process, including the choice of trigger prior to oocyte collection, and certain laboratory procedures. Identification of which of these are predictors of maturation in individual centres enables local protocols to be optimised.

Study design, size, duration: This is a retrospective study of 714 oocyte collections from 661 women between January 2020 to November 2020 treated in a large, single centre in the UK. Subsequent fertilisation on fresh oocytes consisted of 371 IVF and 343 ICSI cycles.

Participants/materials, setting, methods: Patient and treatment data was collected by clinical staff at time of treatment. Either GnRH agonist, hCG or double trigger were administered 36 hours before collection. Prior to ICSI, oocyte maturation was assessed by visualisation of polar body (PB) extrusion. After IVF, the number of 2PNs plus unfertilised oocytes with PB extrusion were assessed. Univariate analyses consisted of Mann-Whitney test, t-test, Fisher's Exact test or ANOVA. Potential predictors were investigated by logistic regression.

Main results and the role of chance: The end point was maturation rate, defined as high (greater or equal to 70%) or low (less than 70%). Factors predictive of a high rate included insemination more than 4 hours after collection. Oocytes inseminated over 4 hours post-collection displayed significantly higher maturation rates than oocytes inseminated less than 2 hours after collection (69% and 61% respectively; P=0.01). Oocytes inseminated between 2-4 hours also had higher maturation than those inseminated less than 2 hours post-collection, but this did not reach significance (67% and 61%, respectively; P=0.06). Further, oocytes fertilised by ICSI had significantly higher maturation than conventional IVF (77% and 67%, respectively, P<0.001). No significant difference in

oocyte maturation between triggers was observed. Similarly, neither age, AMH, a diagnosis of PCOS or number of oocytes collected predicted oocyte maturation in univariate analysis. Logistic regression analysis showed only time between oocyte collection and insemination (aOR 2.12; 95% CI 1.03-4.38; *P*=0.04) to be a significant independent predictor.

Limitations, reasons for caution: Varying means of data collection across clinics and between clinical staff inevitably leads to provision of incomplete data and should be taken into consideration alongside interpretation. Prescription bias of specific triggers to certain patient demographics should be noted.

Wider implications of the findings: Collectively, these results suggest that greater time between oocyte collection and insemination could be recommended to IVF clinics that wish to optimise their oocyte maturation. Triggering final maturation with GnRH agonist versus hCG or dual trigger did not have a significant effect on oocyte maturation when adjusted for confounders.

Trial registration number: not applicable

P-691 How predictive is endometrial thickness for live birth after fresh and frozen-thawed embryo transfer when no cut-off is employed?

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Study question: Does endometrial thickness (EMT) predict live birth (LB) after fresh and frozen-thawed embryo transfer (ET) and is there a lower EMT cut-off for ET?

Summary answer: Once intracavitary pathology and inadvertent progesterone exposure is excluded, EMT is not predictive for LB. EMT is not linearly associated with probability of LB.

What is known already: EMT is commonly used as a marker of endometrial receptivity and in turn, assisted reproductive technology treatment success. ET is often cancelled or postponed if EMT is below an arbitrary cut-off. However, the available evidence on the relationship between EMT and LB rates is conflicting and too dubious to hold such strong stance. An overwhelming majority of the studies on the subject are retrospective, they use different arbitrary cut off values ranging between 6 to 9 mm with heterogeneous stimulation and transfer protocols.

Study design, size, duration: Records of all women who underwent fresh or frozen-thawed ET in Koc University Hospital Assisted Reproduction Unit between October 2016 - August 2019 were retrospectively screened. All women who underwent fresh or frozen-thawed blastocyst transfer during the study period were included. Every woman contributed to the study with only one transfer cycle for each category, i.e., fresh ET and frozen-thawed ET.

Participants/materials, setting, methods: After ruling out endometrial pathology, EMT was measured on the day of ovulation trigger for fresh ET cycles, and on the day of progesterone commencement for frozen-thawed ET. ET was carried out, regardless of EMT, if there was no suspicion of inadvertent progesterone exposure, i.e., due to follicular phase progesterone elevation in fresh or premature ovulation in frozen ET cycles.

Main results and the role of chance: 560 ET cycles, 273 fresh and 287 frozen-thawed, were analyzed. EMT varied from 4mm to 18mm. EMT were similar between women who achieved a LB and who did not after fresh ET [10.5 (9.2 – 12.2) mm and 9 (8 – 11) mm, respectively, p=0.11]. Ovarian stimulation characteristics and proportion of women who received a single embryo were similar (69% vs 68.3%, respectively, p=0.91). Women who achieved a LB was significantly younger than those who did not [35 (32–38) and 37 (33–41), respectively, p<0.01]. Women who had a LB and who did not after frozen-thawed ET had similar EMT of 8.4 (7.4 – 9.7) mm and 9 (8 – 10) mm, respectively (p=0.38). Women who achieved a LB were significantly younger than those who did not [32 (29–35) vs 34 (30–38) years, p=0.04]. The proportion of women who received a single ET was similar between women who achieved a LB and who did not after a FET [86/95 (90.5%) vs 181/192 (94.3%), respectively, p=0.26].

Area under curve values of EMT for predicting LB in fresh, frozen-thawed and all ET were 0.56, 0.47 and 0.52, respectively. EMT and LB rate were not linearly correlated in fresh or frozen-thawed ET cycles.

Limitations, reasons for caution: Although our study is retrospective, no women was denied ET due to EMT in our center. Only patients undergoing ET were included in the analysis, which may introduce bias due to the selection of couples who were competent enough to produce at least one blastocyst fit for transfer.

Wider implications of the findings: Since women with thin endometrium had reasonable chance for LB even in the absence of a cut-off for EMT in this unique dataset, delaying or denying ET for any given EMT value alone does not seem justified. Further studies in which ET is carried out regardless of EMT are needed.

Trial registration number: Not applicable

P-692 Are live birth rate and obstetric outcomes different between immediate and delayed embryo transfers following a freeze-all cycle? A retrospective study combined with a meta-analysis

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Study question: Do livebirth rate (LBR), obstetric and perinatal outcomes vary between frozen embryo transfers (FET) in the first or subsequent menstrual cycles following a freeze-all approach?

Summary answer: Immediate FET has a higher LBR and similar perinatal outcomes than delayed FET. Quantitative synthesis of available literature shows an increased LBR with immediate transfer.

What is known already: Whether FET should be done in the first menstrual cycle following oocyte collection (OC) is controversial and the duration of a possible detrimental effect of supraphysiological sex steroid levels on pregnancy outcome is unknown. A multinational survey centers showed that, 61% of clinicians prefer to wait for a washout period before proceeding to FET, even after a failed fresh embryo transfer. Limited number of studies compared FET in the first menstrual cycle with delayed FET in a subsequent cycle with varying results**.** There is limited data on obstetric outcomes of pregnancies resulting from FET in the first menstrual cycle.

Study design, size, duration: 198 women who underwent a freeze-all cycle followed by FET between July 2017 and June 2020 were included. 119 FET in the first menstrual cycle (<30 days from oocyte collection) and 79 FET in subsequent cycles (>30 days from oocyte collection) were retrospectively compared. MEDLINE was searched on 01 January 2021 using relevant keywords. Cohort studies comparing immediate versus delayed transfer following freeze all cycles were included and quantitative summary for LBR was obtained.

Participants/materials, setting, methods: Freeze-all was undertaken when (i) the woman is deemed to be at high risk for OHSS, (ii)serum progesterone level is >1.5 ng/ml on the day of trigger, (iii)preimplantation genetic testing is planned, (iv)the woman will undergo surgery prior to ET, (v)couple preference.

Main results and the role of chance: Baseline characteristics were similar between the groups except for antral follicle count (22 vs 18, MD= 5, 95% CI= 0 to 8), and number of metaphase-two oocytes (13 vs 10, MD= 3, 95% CI= 1 to 6) all of which were significantly higher in the immediate transfer group. Clinical pregnancy rate (CPR) per ET was similar in two groups (50.4% vs 44.3%, RR= 1.14, 95% CI= 0.84 to 1.54). Miscarriage rate per pregnancy was significantly lower (12.3 vs 31.1, RR= 0.40, 95% CI= 0.19 to 0.84) and LBR per ET was significantly higher (42.9 vs 26.6, RR= 1.61, 95% CI= 1.06 to 2.46) in the immediate transfer group.

Median gestational age at delivery was similar (267.5 (262.5-273) vs 268 (260-271.5) days, MD= 1.00, 95% CI= -4.00 to 5.00). Median birthweight was significantly higher in the delayed transfer group (3520 vs 3195 grams, MD= -300, 95% CI= -660 to -20 grams). Birthweight percentile, height at birth and head circumference were similar between groups.

Literature search revealed 1712 studies from which nine were eligible for quantitative summary. Cumulative risk ratio showed a 10% increase in LBR with immediate transfer compared to delayed transfer (RR=1.10, 95% CI=1.01 – 1.20, I2=67%, 17369 embryo transfers).

Limitations, reasons for caution: Our study is limited by its retrospective design and relatively limited sample size for multivariate analyses. Yet, it is reassuring that the majority of our findings are consistent with previous publications.

Wider implications of the findings: The hypotheses generated by our retrospective findings, i.e., FET in the immediate menstrual cycle resembling

fresh ETs with strong trends towards lower birthweight and lower incidence of preeclampsia is noteworthy for the design of future studies, and these outcomes should be followed and reported.
Trial registration number: None

P-693 Gonadotropin stimulation reduces the implantation and live birth but not the miscarriage rate – a study based on the comparison of stimulated and unstimulated IVF

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Study question: Does gonadotropin stimulation in conventional IVF (cIVF) affect the implantation, miscarriage and live birth rates?
Summary answer: Gonadotropin stimulation negatively affects the implantation and live birth but not the miscarriage rate in IVF treatments.
What is known already: Literature hypothesizes that embryos derived from unstimulated, natural cycle IVF (NC-IVF) have a higher implantation potential compared to embryos from cIVF. In NC-IVF, recruitment of the leading follicle is based on natural selection. Hormonal stimulation might not only affect the embryo but also endometrial function. It's possible to compare outcomes of NC-IVF and cIVF if cIVF is performed without embryo selection, in other words, if only those zygotes, which will be transferred 1-2 days later, are left in culture and all other zygotes are cryopreserved. To test this hypothesis, we compared success rates in NC-IVF and in cIVF.
Study design, size, duration: We performed a cohort study from 2011-2016 including data on IVF cycles with transfer of fresh embryos on day 2-3 at a University based infertility center. Our sample consisted of 640 women with 1482 embryos transferred in 996 cycles. We defined implantation rate as the number of sonograhically detected amniotic sacs per transferred embryos. Data originated from the Swiss ART registry "FIVNAT" and the Bern IVF Cohort and was completed using medical and delivery records.
Participants/materials, setting, methods: We defined NC-IVF as IVF without stimulation of follicular growth and cIVF as IVF with gonadotropin stimulation ≥75 IE/d and >3 retrieved oocytes. We performed zygote, but not embryo selection and transferred embryos on day 2-3. We calculated implantation and live birth per transferred embryo as binary outcomes using bi- and multivariable multilevel logistic regression models accounting for two clusters; the women and the cycle; and adjusting for maternal and infertility characteristics using STATA.
Main results and the role of chance: Age of women (p=0.531), parity (p=0.194) and type of infertility (primary vs secondary) (p=0.463) did not differ between women undergoing NC-IVF or cIVF. In NC-IVF, 468 (31.6%) embryos were transferred, 450 as single, 18 as double transfers. In cIVF, 1014 (68.4%) embryos were transferred, 91 as single, 830 as double and 93 as triple transfers.

Implantation rate was higher in NC-IVF. In NC-IVF 80 (17.1%) and in cIVF 132 (13.0%) embryos developed into an amniotic sac (OR 1.58; 95% CI 1.01-2.46; p=0.042). After adjustment for maternal age (p<0.001), parity (p<0.001), type of infertility (p=0.037), duration of subfertility and indication for IVF, aOR for implantation per transferred embryo increased to 1.87 (95% CI 1.21-2.91; p=0.005).

Miscarriage rate was similar. In NC-IVF and cIVF 25% (n=20; n=33) miscarried and 75% (n=60; n=99) ended in a live birth, respectively (OR 0.91; 95% CI 0.32-2.60; p=0.855; aOR 1.0; 95% CI 0.42-2.36; p=1.000).

Live birth rate per transferred embryo was increased in NC-IVF; 60 of 468 (12.8%) embryos in NC-IVF compared to 99 of 1041 (9.8%) embryos in cIVF resulted in a live birth (OR 1.51; 95% CI 0.92-2.49; p=0.106); and became significantly higher after adjustment (aOR 1.85; 95% CI 1.16-2.95; p=0.010).
Limitations, reasons for caution: This study analyses observational data from a clinic offering NC-IVF and cIVF treatment as equivalent options. NC-IVF is a model for natural fertility and allows us to study the impact of gonadotropins. However, it is not a randomised study and therefore prone to selection bias.
Wider implications of the findings: The study suggests that gonadotropin stimulation might reduce the implantation potential and subsequently live birth rates, by possibly affecting embryo and endometrium quality. Clinicians should consider lower gonadotropin doses for stimulation.
Trial registration number: not applicable

P-694 Natural cycle vs Accumulation of oocytes. Which strategy is better for women with the poor ovarian response (POR)?

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Study question: The aim of this study is to determine whether repetitive natural cycles (Strategy A) or oocyte accumulation (Strategy B) is a more effective strategy.
Summary answer: There was no statistical difference between the strategies however, the number of attempts for successful outcome with strategy A was lower than in strategy B.
What is known already: For populations with poor ovarian response natural cycles (NC) or modified natural cycles (mNC) with minimal stimulation have been implemented as a preferable option as opposed to conventional ovarian stimulation. Due to the development of advanced vitrification techniques, the accumulation of oocytes has become available.

Previous studies (2011, 2013, 2019) suggest that accumulation of oocytes could be a successful alternative to repetitive natural cycles for poor responders aged ≥35 years, showing higher clinical pregnancy rates. Moreover, the embryo-transfer cancellation and miscarriage rate were significantly lower in the oocyte accumulation strategy.
Study design, size, duration: Present retrospective cohort study included a selection of patients with POR treated from 2019-2020, which were divided into 2 strategies. Strategy A included 324 natural cycles or modified natural cycles with successful oocyte retrievals (Female mean age: 36.3 years). The strategy B consisted of 46 cycles with thawed oocytes that were accumulated through cryopreservation in several attempts (average n=2,3) prior in NC or mNC (Female mean age: 37.6 years).
Participants/materials, setting, methods: POR was defined by following criteria: 1) advanced maternal age (≥35 years) or;

2) previous POR (≤3 oocytes with a conventional ovarian stimulation protocol);

3) abnormal ovarian reserve test;

The vitrification of the oocytes was performed using Kuwayama method (Cryotech®, Japan), while thawing was performed using Cryotop method (Kitazato®, Japan). Prior to fertilization, oocytes were cultured for 2 hours in IVF medium (Origio®, Denmark). The unsuccessful transvaginal oocyte retrievals (TVOR) were excluded from both strategies.
Main results and the role of chance: The clinical pregnancy rate per started cycle and per transfer in Strategy A vs Strategy B were 15,4% vs 15,2% and 28.08% vs 20%, respectively. Strategy A seemed to achieve higher rates; however, the difference was not statistically significant (Student's t-test, p <0.05). Throughout all TVORs the rate of successful retrieval was 71.7%. The embryo-transfer cancellation rates in Strategy A vs Strategy B were 38,2% vs 8,69%, respectively. With strategy A the average amount of attempts for successful clinical pregnancy was 1.86. With strategy B the average amount of cryopreservations for oocyte accumulation was 2.3.
Limitations, reasons for caution: The number of patients in strategy B was significantly lower than in strategy A. Larger study with an increased number of samples is necessary to confirm the results. In addition calculation of cost-effectiveness in each strategy. The unsuccessful TVORs were excluded from both strategies, which significantly affects the statistical rates.
Wider implications of the findings: This study might help in developing and selecting more appropriate strategies for women with POR. The findings might help to determine the amount of time and attempts required for a successful outcome for patients aged ≥35. It can also be helpful in regulating the financial part of artificial reproductive technology.
Trial registration number: N/A

P-695 Establishing predictors of the mode of conception in fertility patients presenting with a clinical pregnancy

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Study question: What are the predictors for pregnancies conceived spontaneously (SC), by ovulation induction+/-insemination (OI±IUI) or via In-Vitro Fertilization(IVF), and what proportion of pregnancies were conceived with each method?
Summary answer: Pregnancies were conceived by SC(27.7%), OI±IUI(33%) or IVF(39.2%).Unexplained infertility positively-predicted SC and OI±IUI-conceptions. Male factor-infertility demonstrated the opposite trend, positively predicting IVF. Endometriosis negatively-predicted SC.
What is known already: Spontaneous conception (SC) occurs regularly among infertility patients. Most studies have evaluated predictors of pregnancy among women with infertility who were trying to conceive. Few studies have addressed the role of different factors on the mode of conception in infertility patients who were pregnant. Factors found in some studies to be related with a SC were younger female age, shorter duration of infertility, fewer failed IVF cycles, and diagnosis of unexplained-infertility.
Study design, size, duration: We conducted a retrospective cohort study at a University fertility-center over a six-month period in 2019 and 2020. We reviewed viability scans of 285-patients. Mode of conception was recorded as Spontaneous, OI±IUI, or IVF. Patients' demographics, obstetric and fertility diagnosis as well as base-line hormones and ovarian reserve testing were extracted to calculate predictors for the mode of conception. Pregnancy was defined as an intra-uterine fetal sac on a transvaginal ultrasound in the 1st-trimester.
Participants/materials, setting, methods: Parametric analysis was done using ANOVA and Tukey's post-hoc test. Nonparametric analysis was performed using the chi-square test. Predictors of the mode of conception were calculated by multivariate regression analysis using the variables not in the equation model including the following parameters: male and female age, gravidity, parity, ectopic-pregnancies, infertility diagnosis, baseline serum: FSH, estradiol, TSH, AMH, and AFC. Data is presented as mean ±SD or percentage. P<0.05 was significant. IRB approval was obtained.
Main results and the role of chance: 79 (27.7%) of pregnancies were SC, 94 (33%) resulted from OI±IUI, and 112 (39.2%) from IVF. Demographics didn't differ between the groups including: female age(p=0.06), male age(p=0.79), gravidity (p=0.47), parity(p=0.7), ectopic-pregnancies(p=0.07), baseline serum FSH(p=0.29), estradiol(p=0.65), TSH(p=0.56), AMH(p=0.42), and AFC(p=0.06). Infertility diagnoses differed when comparing SC, OI±IUI and IVF conceptions respectively: Unexplained (22.7%, 22.3%, 15.1%, p=0.03), Male-Factor(MF) (25%, 27.6%, 42.8%, p=0.042), Tubal-factor (2.5%, 2.1%, 13.4, p=0.002) and Ovulation-disorders/PCOS (24%, 32%, 12.5% p=0.002). Endometriosis trended higher in women with IVF (p=0.09).

A positive predictor for SC was unexplained infertility(p=0.0001). A negative predictor was endometriosis(p=0.005). SC was sub-significantly less likely in the presence of MF (p=0.057). Unexplained-infertility was a positive predictor for OI±IUI pregnancies(p=0.047), whereas MF was a negative predictor(p=0.0001). As for IVF-conceptions, MF was a positive predictor(p=0.008), while unexplained-infertility negatively predicted conception by IVF(p=0.018). Ovulation-disorders/PCOS trended lower in women with IVF (p=0.052). While baseline serum estradiol levels were similar between groups (means 194-218pmol/L), multivariate regression showed it to be a predictor for OI±IUI and IVF conceptions. The clinical significance of this finding is not clear. Interestingly, female age and ovarian reserve were not found to predict one type of conception over another. Other possible predictors in the model were not significant.
Limitations, reasons for caution: This retrospective cohort may hide underlying bias. Clinical pregnancies were evaluated and not live birth. Our cohort represents patients that conceived and do not offer information about the entire sub-fertile population that is treated in our center, which is also a strength as it's a novel way of evaluating predictors.
Wider implications of the findings: Among patients that conceived spontaneously, advanced age and ovarian reserve did not play a negative role. Predictors of pregnancy were confirmed as expected with the majority of unexplained infertility conceptions occurring spontaneously or with OI+/-IUI, patients with Male factor infertility often conceived by IVF, and ovulation disorders by OI+/-IUI.
Trial registration number: NA

P-696 The duration of estrogen treatment prior to frozen-blastocyst transfer does not impact live birth rate

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Study question: Does the prolonged duration of oestrogen treatment prior to frozen-blastocyst transfer (FET) affect live birth rate?
Summary answer: Variation in the duration of estrogen treatment prior to frozen-blastocyst transfer does not impact live birth rate.
What is known already: With improvements in cryopreservation techniques and fertility preservation, single embryo transfer policy and the increase in freeze-all cycles, frozen blastocyst transfer (FET) has strongly risen over the last years. Artificial endometrial preparation (AEP) is often used prior to FET. The endometrium is prepared by a sequentially treatment of estrogen and progesterone in order to synchronize endometrium and the embryo development. Whether the duration of progesterone administration before FET is well established, the optimal estrogen treatment duration remains controversial.
Study design, size, duration: All consecutive frozen thawed autologous blastocyst transfer cycles conducted between January 1, 2012 and July 1, 2019 in our University IVF center were included in this retrospective cohort study. We included 2235 single blastocyst FET cycles prepared with hormonal replacement therapy using oral E2 and vaginal progesterone administration in 1376 patients aged from 18 to 43 years.
Participants/materials, setting, methods: Patient's characteristics, stimulation characteristics, FET cycles characteristics and cycles outcomes were anonymously recorded and analyzed. Univariate and multivariate analysis were performed. At first, each FET cycle was analyzed individually and secondly taking into account that some of the patients had undergone several FET, the model considered the number of implanting attempts for each woman.
Main results and the role of chance: We found no significant difference in the mean duration of estradiol administration before frozen embryo transfer between the group live birth versus non-live birth (27.0 ± 5.4 days versus 26.6 ± 5.0 days ; p=*0.11). Endometrial thickness was not significantly different between the 2 groups (8.3 ± 1.7 mm versus 8,2 ± 1,7 mm ; p=0.21). When the duration of estradiol exposure was analyzed in weeks, we observed no difference for the £ 21 days group (OR=0.97 ; IC 0.64–1.47 ; p=0.88), 29-35 days group (OR=0.89 ; IC 0.68–1.16 ; p=0.37) and > 35 days group (OR=0.75 ; IC 0.50–1.15 ; p=0.10) compared to the reference group (22-28 days). After multivariate analysis, the duration of estradiol treatment before frozen embryo transfer did not affect live birth.*
Limitations, reasons for caution: The relatively limited numbers of cycles with more than 35 days or less than 21 days as well as the retrospective design of the study are significant limitations.
Wider implications of the findings: Variation in the duration of estradiol supplementation before progesterone initiation does not impact FET outcomes. We therefore can be reassuring with our patients when E2 treatments need to be extended, allowing flexibility in scheduling the day of transfer.
Trial registration number: not applicable

P-697 screening for adrenocortical and thyroid peroxidase antibodies to look for underlying autoimmune etiologies in women under 35 with idiopathic diminished ovarian reserve

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Study question: Investigate whether screening for autoimmune etiologies is necessary in women with diminished ovarian reserve (DOR) as recommended in the evaluation of premature ovarian insufficiency (POI).
Summary answer: Adrenocortical antibodies (ACA) screening can be performed in the evaluation of women with idiopathic DOR, especially those with a family history of autoimmune disease.

What is known already: Autoimmune disorders are more common in POI than in the general population. The most important association is with autoimmune Addison's disease. Measurement of ACA and / or 21 OH-A is recommended in every POI patients as they appear to be the marker with the highest diagnostic sensitivity for autoimmune POI. Also thyroid peroxidase autoantibodies (TPO-Ab) should be assayed due to the common association between thyroid disease and POI. The underlying etiologies of DOR in young women can be expected to be similar to the etiology of POI since they represent a continuum in the phenotypic expression of premature ovarian aging.

Study design, size, duration: This pilot case-control study was conducted between January 2019 and April 2020. The study group consisted of patients under the age of 35, who was diagnosed with idiopathic DOR by ovarian reserve tests during infertility work up. Controls were patients of the same age range who diagnosed with isolated tubal factor or male infertility and had normal ovarian reserve test results during infertility work up.

Participants/materials, setting, methods: Patients with a history of ovarian surgery, cancer, genetic or autoimmune disease were excluded. Abnormal ovarian reserve tests are defined as antral follicle count < 5 and AMH < 1.2 ng/dl corresponding to group 3 according to POSEIDON criteria. In total, 35 DOR patients and 35 controls were included in the study. ACA and TPO-Ab screening were performed in serum samples using indirect immunofluorescence method. Demographics and family history of autoimmune diseases were also evaluated.

Main results and the role of chance: A higher rate of ACA positivity was detected in the DOR group (34.3%) compare to controls (17.1%), although it was not found to be statistically significant (p=0.101). The incidence of family history of autoimmune diseases in first degree relatives was positively correlated with ACA positivity (p=0.006). In DOR group, autoimmune disease history in the family was significantly higher in ACA (+) patients compared to ACA (-) individuals (p = 0.03). TPO-Ab positivity rates were similar between 2 groups (17.1% vs %20, p=0.75).

Limitations, reasons for caution: Since this is an observational study and also due to the small sample size, a causal conclusion cannot be reached.

Wider implications of the findings: Even if there is no specific treatment option yet for autoimmune ovarian damage, screening for ACA or 21 OH-A may be considered in young women with idiopathic DOR based on knowledge that identification of women with autoimmune POI is clinically important for the identification of subclinical autoimmune Addison's cases.

Trial registration number: non applicable

P-698 Antimüllerian Hormone (AMH) value as a predictive marker of cycle ploidy outcome

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Study question: Do woman with diminished ovarian reserve exhibit poor blastocyst formation and ploidy outcomes, irrespective of age?

Summary answer: Patients with extreme diminished ovarian reserve (AMH≤0.65ng/ml) have a lower chance to have at least one euploid blastocyst compared to their age-related reference population (AMH=1.3-6.25ng/ml).

What is known already: AMH is an established marker of the ovarian reserve for predicting ovarian response to ovarian stimulation and it is strongly correlated with female age.

However, it has been suggested that AMH is not only a quantitative, but also a qualitative biomarker of oocyte/embryo competence. Previous studies show conflicting outcomes as to whether reduced ovarian reserve *per se* is associated with decreased oocyte developmental competence, leading to increased aneuploidy rates in embryos independent of the patient's age.

Study design, size, duration: A retrospective analysis was performed between March 2017 and July 2020 at ART Fertility Clinics (Abu Dhabi) including all couples that were triggered for final oocyte maturation and planned for Preimplantation Genetic Testing for Aneuploidies (PGT-A). Patients were stratified into four age categories [≤30, 31-35, 36-40, >40 years]. For each age category patients were further divided into three AMH groups: ≤0.65ng/ml, 0.65-1.3ng/ml and 1.31-6.25ng/ml (reference group).

Participants/materials, setting, methods: Trophectoderm biopsy samples were subjected to Next Generation Sequencing. AMH serum levels (ng/ml) were determined using the commercial fully automated Elecsys® (Roche) assay. Patients with a Progesterone rise of >1.5ng/ml on the day of final oocyte maturation and patients with AMH values >6.25ng/ml were excluded from the analysis. Per patient that was triggered, the chance to have at least one euploid blastocyst in that cycle, was calculated.

Main results and the role of chance: A total of 1.300 couples were included with an mean maternal age of 35.6±6.2 years, AMH of 2.1 ±1.5ng/ml and body mass index of 27.5±5.0 kg/m2. The chance to have at least one blastocyst biopsied per cycle was affected in all patients with extreme low AMH (≤0.65ng/ml), irrespective of age; ≤30 years: 58.33%-100.00%-94.84% (p<0.001); 31-35 years: 50.00%-74.55%-95.32% (p<0.001); 36-40 years: 56.52%-81.93%-92.56% (p<0.001) and ≥40 years: 38.06%-73.02%-88.24% (p<0.001), for AMH ≤0.65ng/ml, 0.65-1.3ng/ml and 1.31-6.25ng/ml, respectively. In all age categories, patients with AMH values ≤0.65ng/ml had a significantly reduced probability of having a euploid blastocyst compared to the reference group (1.31-6.25ng/ml). For women ≤30 years the chances of getting a euploid blastocyst decreased from 88.89% (n=252) to 41.67% (n=12) (OR 0.01 [0.03-0.30], p<0.001), for 31-35 years from 88.09% (n=235) to 43.75% (n=32) (OR 0.10 [0.05-0.23], p<0.001), for 36-40 years from 77.67% (n=215) to 21.74% (n=69) (OR 0.08 [0.04-0.15], p<0.001) and among women >40 years from 29.42% (n=102) to 6.45% (n=155) (OR 0.16 [0.08-0-36], p<0.001). Woman within AMH range of 0.65-1.3ng/ml presented the same decreased probability of having a euploid blastocyst only when 31-35 (52.73%, n=55) or 36-40 years old (56.63%, n=83) (OR 0.15 [0.08-0.29], p<0.001 and OR 0.37 [0.22-0.64], p<0.001, respectively).

Limitations, reasons for caution: The main limitation of this study is its retrospective design.

Wider implications of the findings: AMH is a clear biomarker of oocyte-embryo competence. Incorporation of AMH-specific counseling recommendations into clinical practice guidelines, could lead to a more informed guidance on cycle ploidy outcomes, rather than age alone.

Trial registration number: not applicable

P-699 Multi-scale study of the architecture, topography and mechanics of the human ovary from prepuberty to menopause: a blueprint for next-generation bioengineering and diagnosis

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Study question: Does the ovarian ECM have a precise and unique biophysical phenotype, specific to each age, from prepuberty to menopause?

Summary answer: Differences between healthy prepubertal, reproductive-age, and menopausal ovarian tissue, unravel and elucidate a unique biophysical phenotype of reproductive-age tissue, bridging biophysics and female fertility.

What is known already: Ovarian engineering has recently emerged to respond to patient needs and offer reliable models for basic research. It has relied on synthetic and natural biomaterials and microfluidics. However, these techniques were designed based on knowledge acquired from 2D cell culture and animal models.

Our lack of information on the human ovary hampers our ability to mimic the main features of this organ, for clinical applications. The complex composition and hierarchical structure of its ECM complicates the design of truly biomimetic constructs, notably: fiber morphology, interstitial and perifollicular fiber orientation, porosity, topography, and viscoelasticity, which all play a role in mechanotransduction.

Study design, size, duration: Ovarian biopsies were taken from prepubertal (mean age [±SD]=7±3 years, n=21), reproductive age (mean age [±SD]=27±5, n=26) and menopausal (mean age [±SD]=61±6 years, n=29) patients after obtaining their informed consent. All participating adult subjects were undergoing laparoscopic surgery for benign gynecological diseases not affecting the ovaries. Prepubertal tissue was derived from young cancer patients scheduled for ovarian cortex cryopreservation as a fertility preservation strategy, before being subjected to acute gonadotoxic cancer treatments.
Participants/materials, setting, methods: All samples were cryopreserved by slow freezing and kept frozen until the day of their analysis. Tissues provided from the same patients (n=5 per age group) were investigated by scanning electron microscopy (SEM) (fiber, pore and topography analyses) and atomic force microscopy (AFM). A larger number of paraffin-fixed biopsies (prepubertal, n=16, reproductive-age, n=21, and menopausal, n=24) obtained from the biobank of St-Luc's Hospital were used to conduct computed fiber orientation analysis.
Main results and the role of chance: Our results revealed a unique ECM architecture at reproductive age, where fibers of intermediate diameter are assembled into thickest bundles compared to prepubertal and menopausal tissues($p<0.0001$). Indeed, during prepuberty the bundles assemble into a tight network with high number of small pores while reproductive-age ovary gain more porosity($p<0.0001$). However, at menopause tissue pore number and area change significantly($p<0.001$). These pore geometry and distribution changes contribute to diffusion and access of key molecules to/from cells, which can be translated into changes in permeability and molecule selectivity with age. Fiber directionality around follicle borders at preantral stages revealed that before and after puberty, secondary follicles appear to modify their microenvironment arrangement locally compared to follicles at earlier stages of development ($p<0.01$), by reorienting the majority of collagen fibers below 50°.This could indicate that follicles at this stage require higher fiber contact and adhesion signaling to complete their development and maturation towards ovulation. AFM evidenced a relatively rigid ovarian tissue at prepuberty, softening significantly at reproductive age, then stiffening considerably upon menopause. These differences($p<0.01$) are not only structure-dependent, but also related to biochemical differences in ECM composition, as previously demonstrated in our follow-up of variations in elastic matrisome components from prepuberty to menopause.
Limitations, reasons for caution: The samples represent single time points from each age group which could present limitations, since following ovary dynamics from prepuberty to menopause in the same patient is not feasible.
Wider implications of the findings: Our study provides the first conclusive proof of a link between ECM biophysics and fertility by comparing different stages of ovarian transformation related to a woman's reproductive life, which will oriente new strategies for infertility prognoses based on ECM biophysics and may become a blueprint for designing functional engineered ovaries.
Trial registration number: Not Applicable

P-700 Increased progesterone to mature oocyte index is associated with lower top-quality embryo rate in GnRH antagonist protocols

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Study question: Do progesterone elevation (PE) on trigger day and progesterone to mature oocyte index (PMOI) affect embryo quality and the chance of live birth?
Summary answer: The top-quality embryo rate is decreased by increasing PMOI, but it has no association with absolute serum progesterone levels.
What is known already: Progesterone elevation have been reported to significantly decrease pregnancy and implantation rates. The main mechanism of this adverse effect is mainly related to an asynchrony between the endometrium and the embryo. Many of the previous studies have failed to show a significant impact of PE on embryo quality and the success of subsequent frozen-thawed embryo transfer (FET) cycle. However, PMOI was suggested to be more predictive than PE of ART outcome and might be associated with embryo quality.
Study design, size, duration: A single-centre retrospective cohort study was conducted. All FET cycles performed in a university hospital infertility centre between January 2016 and December 2019 were reviewed. A total of 44 patients who had PE (>1.5 ng/ml) on trigger day and 134 patients who did not have PE were assessed.
Participants/materials, setting, methods: The study group consisted of patients who had PE (>1.5 ng/mL) during fresh COS cycle and the control group consisted of patients who did not have PE. In addition to effect of PE on subsequent FET cycle outcome, an association between PMOI and embryo quality was assessed. The threshold level to define increased PMOI (>0.12 ng/ml) was calculated as the median level of the whole study cohort.
Main results and the role of chance: The mean ages of the study and control groups were 30.4±5.4 years and 31.1±5.6 years, respectively (P=0.413). Although the number of oocytes collected and MII oocytes were significantly higher in patients with PE, the total number of frozen embryos were similar between the groups. There were no significant differences concerning the outcome measures including live birth rate in the subsequent FET cycle between participants with and without PE (27.3% vs. 23.9%, respectively; P=0.652). The rate of top-quality embryos was similar between participants with and without PE (43% vs. 52%, respectively; P=0.370). However, the rate of top-quality embryos was significantly lower in cycles with PMOI>0.12 ng/ml than in cycles PMOI<0.12 ng/ml (42% vs. 56%, respectively; P=0.027).
Limitations, reasons for caution: The retrospective design and the small sample size derived from a single institution.
Wider implications of the findings: Increased PMOI, which is associated to lower top-quality embryo rate, may in turn result in diminished cumulative live birth rate.
Trial registration number: not applicable

P-701 Fresh vs frozen PGT-A cycles in donor oocyte recipients

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Study question: For donor oocyte recipients, are clinical outcomes superior for fresh versus frozen euploid embryos?
Summary answer: Among donor oocyte recipients receiving euploid embryos, fresh embryos are associated with superior clinical outcomes when compared with frozen embryos.
What is known already: A recent large retrospective cohort national registry study reported that among donor oocyte recipients, fresh embryos were associated with better clinical outcomes when compared with frozen embryos. This finding contrast with data from autologous oocytes. Since embryo quality at embryo transfer (ET) may introduce a significant confounder, the additional analysis of recipients receiving only euploid embryos may add important information on the subject.
Study design, size, duration: Retrospective cohort analysis of PGT-A IVF-cycles of women using donor oocytes resulting in fresh blastocyst ET compared to the first frozen blastocyst ET from freeze-all cycles between 2014 and 2020 at Instituto Bernabeu, Alicante, Spain. A total of 349 donor oocyte cycles were analyzed, in which 211 were fresh and 138 were frozen ETs. Thawed oocytes were not excluded.
Participants/materials, setting, methods: Clinical pregnancy (gestational sac plus embryo heart beating at 6-7 weeks), was the primary outcome measure. Secondary outcomes included pregnancy and early pregnancy loss rate. aCGH platform tested the embryos transferred in fresh whereas either aCGH or NGS platforms were used for embryos submitted to elective frozen cycles. Vitrification was used as cryopreservation technique. Fresh transfers were performed in artificial cycles. Different types of endometrial preparations were used for FET in the study.
Main results and the role of chance: Recipients in the fresh group were significantly younger and had more embryos transferred compared to the frozen group (41.3 vs 42.5 and 1.2 vs 1.1, respectively). More clinical pregnancies were observed in the fresh compared to the frozen group (108/211 versus 54/138, respectively, odds ratio (ODR) 1.63 [95% CI 1.05–2.52]; p=0.02). Pregnancy rates were also higher in the fresh compared to the frozen group (128/211 versus 63/138, respectively, odds ratio (ODR) 1.83 [95% CI 1.18–2.83]; p=0.005). Early pregnancy losses were similar in both groups (p=0.2).

Limitations, reasons for caution: Implantation failure and abnormal male tests were the most frequent indications for PGT-A. Because of the observational nature of the results in this limited sample size, a cause–effect relationship should not be assumed; evidence from larger well-designed randomized control trials is still required before clinical advice can be suggested.
Wider implications of the findings: When PGT-A analysis is deemed to be necessary in oocyte recipients, cryopreservation may have an adverse impact on IVF outcomes. Future studies exploring ET in natural vs artificial cycles are warranted to further isolate the impact of vitrification and the uterine environment on IVF outcomes.
Trial registration number: Not applicable

P-702 The effect of body weight on assisted reproduction treatment: clinical and perinatal outcomes

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Study question: Does body weight have any effect on clinical and perinatal outcomes in assisted reproduction techniques (ART)?
Summary answer: Obesity and overweight were associated with smaller oocytes retrieved and mature number, tendency to minor pregnancy rates, and with a greater chance of macrosomic newborns.
What is known already: The body mass index (BMI) is an international measure to categorize population regarding body weight. Overweight and obesity have an established negative impact on female fertility, especially due to chronic anovulation. However, studies are inconsistent regarding body weight and ART clinical and perinatal outcomes. Some say there is no difference, others show a little or unfavorable outcomes in overweight and obese patients.
Study design, size, duration: Retrospective cohort study performed at an assisted reproductive clinic. A total of 2296 follicle stimulation cycles were included, from 1686 patients, which resulted in 2278 embryo transfers (ET). Both fresh (1942) and vitrified (354) ET cycles were included in the study. The data refers to a period from 2013- 2020 and were collected from electronic records.
Participants/materials, setting, methods: Sample was divided into groups, according to BMI (kg/m²): Group 01 (<18.5, n=30 cycles); Group 02 (18.5-24.9, n=1630 cycles); Group 03 (25-29.9, n=459 cycles) and Group 04 (≥30, n=177 cycles). Data were presented as mean±SD, median (interquartile range), or percentage. ANOVA and Chi-square tests were applied, considering $p<0.05$. Multiple logistic regression and generalized estimating equations were performed to consider patients and cycles.
Main results and the role of chance: The mean maternal age was 35.71±3.5 years old. A statistically significant difference was observed in retrieved oocytes and mature oocytes number (MII) when groups 01 and 02 were put together (G01+G02) and compared to groups 03 and 04: (8.8 [8.5-9.2] vs 7.9 [7.3-8.6] vs. 7.2 [5.9-8.4], p=0.005) and (6.7 [6.4-7] vs 6 [5.5-6.5] vs. 5.3 [4.3-6.3], p=0.003), respectively. A significant linear tendency to minor pregnancy rates with higher BMI (p=0.038), with no significant difference in pregnancy rates was found between the four groups (52.6% vs. 47.9% vs. 46.7% vs. 36.3%, p=0.124). There was no significant difference in cumulative pregnancy, live birth rate, fertilization and implantation rates between groups. Group 04 showed a higher, but not significant, prevalence of macrosomic newborns (p=0.110). No statistical differences regarding any other clinical and perinatal outcomes were found (prematurity, intensive care unit admission, congenital malformations, Apgar index, newborn percentile, gestational age and birthweight.
Limitations, reasons for caution: This is a retrospective study with a limited number of patients. Also there was no information on patients' weight gain throughout pregnancy, and others clinical pregnancy diseases that could affect perinatal outcomes.
Wider implications of the findings: The study presented that the higher the weight, there seems to be a tendency towards worse outcomes of ART, especially regarding retrieved oocytes and mature oocytes number. Also, the study draws attention to the possible relationship between obesity and perinatal outcomes, also seen in spontaneous pregnancies.
Trial registration number: not applicable

P-703 Association of DENND1A and THADA gene variants with polycystic ovary syndrome in women of Arab ethnicity

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Study question: Are THADA and DENND1A gene variants associated with PCOS in Arab populations suggesting that they are likely to be important in the etiology of PCOS regardless of ethnicity.
Summary answer: All SNPs in DENND1A and THADA found in PCOS cases and controls in our study are different from those reported from other ethnicities.
What is known already: Polycystic ovary syndrome (PCOS) is a complex endocrine disorder with environmental and genetic factors contributing to its etiology. PCOS is the commonest cause of female infertility and is associated with type2 diabetes mellitus and cardiovascular disease. Genome-wide association study (GWAS) of PCOS in Chinese women identified reproducible PCOS susceptibility loci mapping to LHCGR, THADA, and DENNDIA. THADA and DENND1A were reported to be associated with PCOS in European populations suggesting that they are likely to be important in the etiology of PCOS regardless of ethnicity. However the impact of these loci on PCOS in other ethnicities remains to be determined.
Study design, size, duration: A sample size of 49 cases and 49 controls was estimated for a significance level of 5% at 60% power, with minor allele frequencies of 12% and 1% in cases and controls respectively with a 1:1 ratio. We conducted a prospective study on 50 PCOS cases and 50 control Omani females from the Gynecology clinic at the Sultan Qaboos University Hospital, a tertiary care hospital in Oman from 2019 to 2020. Participants/materials, setting, methods: Cases fulfilled the Rotterdam criteria for PCOS. Patients with genetic disorders, congenital adrenal hyperplasia, androgen secreting tumors, Cushing syndrome, and hyperprolactinemia were excluded from this study. Data collected included history, physical examination and laboratory test results. Gene Panel Exome Sequencing was conducted on DNA extracted from blood samples for THADA and DENND1A and data analyzed using ANNOVAR software. Statistical analysis was performed using SPSS for Descriptive statistics, Chi square test and Correlation analysis.
Main results and the role of chance: The highest frequency variants in DENND1A gene among Omani women were: rs2491348, rs2808409, rs2797946, rs2808411 and rs10739633. The highest frequency variants in THADA were: rs11900952, rs4337518, rs1158411, rs11893207 and rs13021894. All SNPs in DENND1A and THADA found in PCOS cases and controls in our study are different from those reported from other ethnicities. There was a significant association of DENND1A rs2808411 with Hirsutism and high TSH (p value ≤ 0.05). For THADA there was a mild negative correlation between TSH levels and the occurrence of rs33979934 among PCOS cases (p value ≤ 0.05). No significant relationship was found between other hormonal parameters and the occurrence of other selected THADA variants among cases. There was a mild negative correlation between High Density lipoprotein (HDL), total Cholesterol levels and the occurrence of rs33979934 among PCOS cases (p value ≤ 0.05). No significant relationship was found between hormonal or biochemical parameters and the occurrence of variants among the controls.
Limitations, reasons for caution: The limitation of this study were the small sample size and that the study was conducted in a single center.
Wider implications of the findings: Further analysis is needed to establish a cause-and-effect relationship between THADA, DENND1A genes and PCOS among women of Arab ethnicity.
Trial registration number: IG/MED/OBGY/19/01

P-704 Does the type of ovarian stimulation scheme and dose matter when calculating the amount of gonadotropins used to obtain an egg?

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Study question: Is the controlled ovarian stimulation protocol important to determine the ovarian sensitivity index (number of oocytes retrieved / total gonadotropin dose)?
Summary answer: OSI in an interesting tool in estimating the ovarian sensitivity to exogenous gonadotropins and can be adjusted when different kinds of COS procotols are used.
What is known already: In our country, Argentina, as the fertility treatments are financed by different health care insurance companies, COS protocols may vary depending on the drugs provided. The objective of our study is to assess the oocyte retrieval number in patients undergoing COS, for IVF or fertility preservation (egg freezing), on the basis of the kind of protocol and the average gonadotropin dose.
Study design, size, duration: This is a retrospective and descriptive study in which 684 cycles, performed between November 2018 and March 2020, were evaluated. Patients underwent COS for IVF or fertility preservation purposes.
Participants/materials, setting, methods: COS protocols were analysed including different parameters such as: number of mature follicles (17mm or more) the trigger day, number of oocytes retrieved, number of M2 retrieved, follicle to oocyte index (FOI), and the kind of protocol.Each of these groups were divided into four groups depending on the protocol used. Group A: FSHr; Group B: FSHr/LHr; Group C: FSHr + HMG; Group D: Clomiphene citrate + HMG. Oocyte donors and incomplete medical records were excluded
Main results and the role of chance: The total number of cycles analysed was 684. There were 135(19.7%) cycles in the under 35 age group; 340(42%) in the 35 to 39 group; 209(38%) in the older than 40 group. They were subdivided in Group A: 54(6.67%) cycles; Group B 93(11.11%) cycles; Group C 465(71.72%); Group D 72(10.5%). A total number of 11351 oocytes were retrieved, with a mean average of 4.6 +- 2.5 SD per patient. Assessment of dose of gonadotropin per oocyte retrieved in each different group: GROUP A 360IU+-38 SD; GROUP B 390IU+-43 SD; GROUP C 375IU +-44 SD; GROUP D 479+-36 SD.
Limitations, reasons for caution: This is a retrospective study. Further prospective studies and a higher sample size are needed to confirm whether the OSI is a useful tool to adjust gonadotropins doses related to an specific COS protocol and improve the oocyte pick up.
Wider implications of the findings: The main conclusion obtained was that the average dose of gonadotropin needed to obtain one mature oocyte was 360 to 390 IU. The HMG and clomiphene citrate protocol needed the highest dose of gonadotropin (479 IU) but no significant stadistical differences were observed.
Trial registration number: NA

P-705 Basal FSH and AMH profiles impact embryo morphokinetics in a maternal age-dependent manner

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Study question: Do maternal AMH and basal FSH profiles impact embryo morphokinetics and does this relationship change with maternal age?
Summary answer: Embryo morphokinetics varies with basal FSH and AMH levels and this relationship changes in advanced maternal age (AMA).
What is known already: Basal FSH and AMH levels have been utilised as markers of ovarian reserve/response and IVF/ICSI outcomes. Basal FSH better reflects post-IVF/ICSI live birth occurrence in pre-AMA patients, while AMH appears more robust in AMA patients. Whilst plasma AMH levels reflect oocyte yield, recent data suggest that plasma basal FSH and intrafollicular AMH levels specifically reflect oocyte quality. Oocyte and embryo developmental competence is associated with faster fertilisation and embryonic morphokinetics. The assessment of the relationship between developmental morphokinetics and maternal basal FSH and AMH plasma profiles shall contribute for a better understanding of their roles as fertility markers and regulators.
Study design, size, duration: Retrospective cohort study including 1961 first autologous ICSI cycles performed from 2014 to 2020, providing 10774 embryos grouped according to maternal AMH and basal FSH levels in: CF [concordant favourable; AMH>1 (ng/mL), FSH≤10 (IU/L); n=8055)]; DFA (discordant with favourable AMH; AMH>1, FSH>10; n=768); DFF (discordant with favourable FSH; AMH≤1, FSH≤10; n=1362) and CU (concordant unfavourable; AMH≤1, FSH>10; n=589). Morphokinetic parameters were compared among groups in total, ≤35 (pre-AMA) and >35 (AMA) years old patients, separately.
Participants/materials, setting, methods: Patients aged 20 to 45 with FSH and AMH levels measured on menstrual cycle day 2 underwent ovarian stimulation, ovum pick-up and ICSI. Embryos were cultured in a time-lapse incubator (Embryoscope). Fertilisation and cleavage morphokinetic parameters [tPNa (time of pronuclei appearance, tPNf (time of pronuclei fading), t2, t3, t4, t5 and t8] were annotated and compared among AMH/FSH groups with the Kruskal-Wallis nonparametric test, followed by a post hoc multiple comparison with Bonferroni correction.
Main results and the role of chance: In overall embryos, tPNa, tPNf, t2 and t3 varied between AMH/FSH groups. CF were faster than CU embryos for all these parameters [(mean±SEM; hours); tPNa: 6.8±0.02 *vs.* 7.1±0.08; tPNf: 24.13±0.04 *vs.* 24.49±0.14; t2: 27.05±0.05 *vs.* 27.36±0.16; t3: 36.94±0.07 *vs.* 37.54±0.22 (p<0.001)]. In addition, CF were faster than DFA (p<0.001), but not than DFF embryos, for tPNf (CF: 24.13±0.04; DFF: 24.23±0.14; DFA: 24.57±0.14) and t2 (CF: 27.05±0.05; DFF: 27.22±0.12, DFA: 27.42±0.15). In AMA patients, faster morphokinetics was observed when one or both hormonal values were favourable; tPNf, t2 and t3 were reached earlier in CF compared to CU (tPNf: 24.17±0.05 *vs.* 24.52±0.15; t2: 27.13±0.06 *vs.* 27.43±0.17; t3: 37.09±0.08 *vs.* 37.63±0.24; p<0.05), but not to DFF (tPNf: 24.22±0.12; t2: 27.27±0.14, t3: 36.92±0.19) or DFA embryos (tPNf: 24.39±0.14; t2: 27.34±0.17, t3: 37.26±0.23). Differently, in pre-AMA patients, faster morphokinetics was associated with favourable basal FSH regardless of AMH levels; tPNa and tPNf were reached earlier in CF compared to DFA and CU (p<0.005), but not to DFF embryos (tPNA: 6.68±0.03, 6.96±0.13, 7.13±0.14, 7.12±0.19; tPNf: 24.05±0.07, 24.27±0.21, 25.14±0.34, 24.37±0.36; for CF, DFF, DFA and CU, respectively).
Limitations, reasons for caution: Our study is subjected to the intrinsic limitations of a retrospective analysis and the results could have been affected by variables that were not controlled for.
Wider implications of the findings: The findings suggest that lower basal FSH levels are associated with faster early morphokinetics likely reflecting superior oocyte quality in pre-AMA patients. The present data may contribute to improve ART prognostic strategies and provide valuable clues for a better understanding of hormonal regulation of oocyte developmental competence.
Trial registration number: not applicable

P-706 Impaired fibrinolysis during estrogen substitution in relation to frozen-thawed embryo transfer

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Study question: Is the clot lysis time prolonged in women undergoing estrogen substitution in artificial cycle during frozen-thawed embryo transfer (AC-FET)?
Summary answer: Women receiving AC-FET have a prolonged clot lysis time that could result in increased venous thromboembolic risk during estrogen substitution.
What is known already: High doses of estrogen are used for women treated with AC-FET; this in contrast to women treated with natural cycle frozen-thawed embryo transfer (NC-FET). Based on previous research on hormone replacement therapy in other settings, estrogen substitution is probably associated with an increased risk of thromboembolism. Moreover, it has formerly been shown that pregnant women followed assisted reproductive technology (ART) treatment as compared to natural fertilization, has an increased risk of thrombosis. However, changes in fibrinolysis has never been examined in women undergoing estrogen substitution during treatment with AC-FET.

Study design, size, duration: Prospective cohort study of women receiving AC-FET with oestrogen/progesterone substitution or NC-FET. Blood samples were obtained four times: 1) prior to hormone substitution (baseline), 2) confirmation of biochemical pregnancy, 3) gestational week 8 and 4) gestational week 13 (5 weeks after cessation of hormone substitution). Inclusion criteria: women aged > 18 years assigned for FET. Exclusion criteria: egg donor recipients, known bleeding disorders, indication for thromboprophylaxis and treatment with anti-platelet medication or non-steroid-anti-inflammatory drugs.

Participants/materials, setting, methods: We included women at the Department of Obstetrics and Gynaecology, Horsens Fertility Clinic, Denmark, from August 2019 – November 2020. In total, 34 participants were included: 19 women treated with AC-FET and 15 women receiving NC-FET. We examined fibrinolysis measured by a dynamic fibrin clot lysis assay that can assess the capacity for fibrin formation and fibrinolysis. This detailed information of the fibrinolytic activity are used as a surrogat marker of thromboembolic risk.

Main results and the role of chance: Our results showed a significantly longer lysis time (908 ± 234 vs 1157 ± 218) ($p < 0.001$) within the AC-FET group after hormone substitution compared to baseline. Moreover, we found a higher area under the curve (AUC) (919 ± 305 vs 1167 ± 391) ($p = 0.006$) within the AC-FET group. However, we observed no changes in mean lag phase or maximum absorbency after estrogen substitution within the AC-FET group. Since we observed a significantly higher AUC within the AC-FET group after estrogen substitution, this is probably due to the prolonged lysis time, indicating hypofibrinolysis. No significant changes was found comparing the NC-FET group with the AC-FET group.

Limitations, reasons for caution: Our data are based on a small study population. Additionally, we cannot exclude that the coagulation parameters could be affected by estrogen prior to study enrollment as we had no wash out period.

Wider implications of the findings: Our findings indicate prothrombotic changes in the AC-FET group. It is relevant to individually consider the indication for AC-FET and restrict the use of unnecessary hormone exposure. These data should be followed by a populations-based study to clarify how this potentially increased venous thromboembolic risk will manifest itself clinically.

Trial registration number: NCT04359576

P-707 Does the dose or type of gonadotropin affect the reproductive outcomes of poor responders undergoing modified natural cycle IVF (MNC-IVF)?

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Study question: Does the dose or type of gonadotropin affect the reproductive outcomes of poor responders undergoing MNC-IVF?

Summary answer: Neither the type nor the dose of gonadotropins affects the reproductive outcomes of poor responders undergoing MNC-IVF.

What is known already: Poor ovarian response (POR) to ovarian stimulation remains a major therapeutic challenge in routine IVF practice, because of the association with low live birth rates and high cancellation rates. Although high doses of gonadotropins are traditionally used to stimulate the ovaries in women with predicted POR, MNC-IVF has been proposed as a mild-approach alternative in this population. Typically, the MNC protocol includes GnRH-antagonists to avoid premature ovulation and gonadotropin add-back stimulation at the late follicular phase. However, evidence is sparse, and there is no consensus regarding a specific dose or type of gonadotropins in this mild stimulation protocol.

Study design, size, duration: This is a retrospective cohort study including patients attending a tertiary referral University Hospital from 1st January 2017 until 1st March 2020.

Participants/materials, setting, methods: All women who underwent MNC-IVF in our center were included. Gonadotropins [recombinant FSH (rFSH), urinary FSH (uFSH) or highly purified human menopausal gonadotrophin (hp-hMG)] were started when a follicle with a mean diameter of 12-14 mm was observed on ultrasound scan, followed by GnRH antagonists (0.25mg/day) from the next day onwards. Mature oocytes were inseminated using ICSI.

Main results and the role of chance: In total, 484 patients undergoing 1398 cycles were included. Mean (SD) age and serum AMH were 38.2 (3.7) years and 0.46 (0.78) ng/ml, respectively. The daily dose of gonadotropins was either <75 IU/d [11/1398 (0.8%)] or 75 to <100 IU/d [1303/1398 (93.2%)] or ≥ 100 IU/d [84/1398 (6%)]. Patients were stimulated with: rFSH [251/1398 (18%)], uFSH [45/1398 (3.2%)] or hp-hMG [1102/1398 (78.8%)]. Biochemical and clinical pregnancy rates were 142/1398 (10.1%) and 119/1398 (8.5%). Live birth was achieved in 80/1398 (5.7%) of cycles. Live birth rates (LBR) were similar between the different type and doses of gonadotropins (p-value 0.3 and 0.51, respectively). The GEE multivariate regression analysis adjusting for relevant confounders (age, BMI, number of MII oocytes) showed that the type of treatment strategy (rFSH/uFSH/hp-hMG) and the dose of gonadotropins were not significantly associated with LBR (coefficient 0.01 and -0.02, p value 0.09 and 0.3, respectively).

Limitations, reasons for caution: The main limitation is the retrospective design of our study, with an inherent risk of bias.

Wider implications of the findings: This is the first and largest study evaluating MNC-IVF protocol modalities. Our data demonstrate that any type of gonadotropin can be used and there is no benefit from daily doses beyond 75IU.

Trial registration number: N/A

P-708 Comparison of reproductive outcomes after progestin-primed ovarian stimulation with dydrogesterone versus cetrorelix to inhibit spontaneous ovulation in oocyte donation

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Study question: Is dydrogesterone (DYG) equivalent compared to cetrorelix with respect to clinical pregnancy rate, ongoing pregnancy rate and live birth rate in oocyte donation (OD) cycles?

Summary answer: DYG is comparable to cetrorelix in terms of clinical pregnancy, but higher rates of ongoing pregnancy and live birth were observed in the DYG group

What is known already: Progestin-primed ovarian stimulation (PPOS) is an ovarian stimulation regimen based on a freeze-all strategy using progestin as an alternative to GnRH analog for suppressing a premature LH surge. DYG is an oral progestin that has been studied in PPOS protocols.

Published reports indicate that length of ovarian stimulation, dose of gonadotrophin needed and number of MII retrieved from PPOS cycles are comparable to short protocol of GnRH agonists during OD cycles. However, while some studies noted no differences in terms of live births, worse pregnancy rates have been reported in recipients of oocytes from PPOS cycles compared to GnRH antagonists.

Study design, size, duration: Prospective controlled study to assess the reproductive outcomes of OD recipients in which the donors were subjected to the DYG protocol (20mg/day) compared with those subjected to the short protocol with cetrorelix (0.25 mg/day) from Day 7 or since a leading follicle reached 14 mm. The OD cycles were triggered with triptoreline acetate and the trigger criterion was ≥3 follicles of diameter >18mm.

Participants/materials, setting, methods: 202 oocyte donors were included, 92 under DYG and 110 under cetrorelix. The study was performed in a private infertility center between January 2017 and December 2020.

The main outcome included the rates of clinical pregnancy, ongoing pregnancy and live births. Secondary outcomes included the number of oocytes retrieved, number of MII, fertilization rate, length of stimulation and total gonadotropin dose. Differences were tested using a Student's t-test or a Chi2 test, as appropriate.

Main results and the role of chance: Compared to antagonist cycles, cycles under DYG had fewer days of stimulation (9.9 ± 0.9 vs. 10.8 ± 1.1, p<.001) and a lower total gonadotropin dose (1654 ± 402.4 IU vs. 1844 ± 422 IU, p<.001). The number of MII retrieved was no different: 16.9 (SD 6.2) with DYG and 15.4 (SD 5.8) with cetrorelix (p=0.072). Recipients and embryo transfer (ET) characteristics were also similar between groups. The mean number of MII assigned to each recipients was 6.7 (SD 1.8) in DYG and 6.6 (SD 1.7) in cetrorelix (P=0.446). The fertilization rate was 66.2% in DYG versus 67.6% in cetrorelix (P=0.68). Regarding the reproductive outcomes, the overall clinical pregnancy

rate in DYG group (65/87: 74.7%) and cetrorelix group (66/104: 63.4%) (p=0.118) was similar. Meanwhile, the DYG group compared to cetrorelix group had higher rates of ongoing pregnancy (63.2% vs 45.1%; p=0.014) and live births (54,9% vs 37.8%; p=0.040).
Limitations, reasons for caution: These results should be evaluated with caution. The limitations of this study include the limited number of participants enrolled and the limited data on pregnancy outcomes. A randomized controlled trial is necessary to provide more evidence on the efficacy of the DYG protocol.
Wider implications of the findings: The efficacy of PPOS protocol compared to GnRH-antagonist protocol in terms of reproductive outcomes has been little studied. PPOS using DYG yields comparable clinical pregnancy rates compared to cetrorelix in OD cycles. The differences found regarding the rates of ongoing pregnancy and live births should be further investigated.
Trial registration number: Not applicable

P-709 Dual stimulation in-vitro-maturation (Duostim IVM) for overcoming oocyte maturation arrest, resulting in embryo transfer and livebirth

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Study question: Does luteal phase followed by follicular phase letrozole priming and dual oocyte retrieval for in-vitro maturation (IVM) overcome oocyte maturation arrest (OMA)?
Summary answer: Oocyte maturation, fertilization,embryo cryopreservation and livebirth can be achieved with letrozole priming IVM in rare cases of OMA.
What is known already: OMA is an intractable problem resulting in only immature oocytes being collected and to date no succesful treatment exists. Attempts to mature oocytes collected in stimulated IVF cycles with OMA have so far failed. Cases with OMA can be due to intrinsic oocyte defects, intrafollicular factors or resistance to stimulation.
Study design, size, duration: Six women with OMA in ≥2 prior stimulated IVF cycles were treated between March 2019 and December 2020.
Participants/materials, setting, methods: Participants had total of 18 (range 2 - 6) prior IVF cycles yielding only 166 immature oocytes. Letrozole 5mg was given days 15-18 of ovulatory cycle; SC decapeptyl 0.1mg trigger given at follicles 12 mm, 38 hours<OPU. After menstruation, letrozole 5mg days 3-7; SChCG 250ug when follicles=12 mm 38 hours<OPU. After in-vitro-maturation oocytes reaching MII were fertilized. Embryos from luteal collection were frozen and fresh embryo transfer was attempted after follicular phase collection.
Main results and the role of chance: Six women underwent DuoStim IVM, median (quartiles) 3.5 (0 - 9) GV and 0.5 (0 - 2) MI oocytes were collected from luteal phase OC and 0 (0 - 0) GV and 2(0 – 4.5) MI oocytes were collected from follicular phase OC. They had a total of 166 immature oocytes collected in prior IVF cycles. There were no MII oocytes at the time of collection in any cycles.0 (0 – 3.5) oocytes matured from luteal phase OC and 1 (0 – 4) from follicular phase OC. 0 (0 – 1.5) embryos were available from luteal phase and 0 (0 - 2) from follicular phase OC.Two subjects (29 and 33 years old) underwent fresh DET and the 29 year old with 2 previous failed IVF cycles achieved a livebirth (50% per ET and 16.7% per started cycle). None of the women who did not have an embryo for fresh transfer from the follicular phase collection had an embryo from the luteal phase collection. The same 29 year old has 2 luteal phase and 2 more follicular phase embryos vitrified.
Limitations, reasons for caution: OMA is a rare condition with a variety of etiologies. Different etiologies can require different managements.
Wider implications of the findings: It may be possible to overcome OMA with letrozole IVM in rare cases. This case is the first recorded live birth. The value of dual stimulation overcoming OMA remains uncertain.
Trial registration number: This study is approved by the local ethical commitee of Medicana Samsun International Hospital by a Grant number of 02/05.02.2020: registration is not required due to retrospective status

P-710 The possible relation between kisspeptin and apoptosis on granulosa cells of PCOS patients

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Study question: Does kisspeptin administration affect the apoptotic factors of granulosa cells of Polycystic Ovary Syndrome (PCOS) patients?
Summary answer: Kisspeptin administration significantly increased apoptosis related factors.
What is known already: Kisspeptin plays a critical role in central gonadotropin secretion by acting on GnRH neurons in the hypothalamus. Kisspeptin receptors are also expressed on the ovaries. Kisspeptin-54 has recently been known as a prominent agent in oocyte maturation. Although there are many studies on the possible roles of kisspeptin administration in vivo on hypothalamic gonadotropin secretion and ovarian function and the possible roles of these effects in the pathogenesis of PCOS, there is not enough information about the effect of in vitro application of kisspeptin on ovarian function and conditions that lead to the development of PCOS.
Study design, size, duration: This basic research study was an in vitro experimental approach involving the use of granulosa cell from a PCOS and normal cases between July to December in 2020. 32 women were included in both control and PCOS groups.
Participants/materials, setting, methods: Women with (n=16) and without (n=16) PCOS were included in the study. Granulosa cells, follicular fluids and blood samples were collected on oocyte pick-up day. Cells were isolated from follicular fluids. Kisspeptin-54 levels were determined by ELISA in serum and follicle fluids. Also, FSH and LH levels were determined in serum. Kisspeptin-54 administrated in vitro to the half of the samples. Fluorescence spectrophotometer and laser scanning confocal microscope were used to evaluate calcium concentrations
Main results and the role of chance: The serum kisspeptin level was found significantly higher in PCOS patients compared to the control group ($p<0.05$). Also, the level of kisspeptin in follicular fluid was statistically significantly higher in PCOS patients compared to the control group ($p<0.05$). The mean serum FSH level of PCOS patients was found to be significantly lower than the control group ($p<0.05$). Furthermore, the mean serum LH level of PCOS patients was significantly higher than control groups ($p<0.05$). The intracellular calcium concentration in granulosa cells obtained from PCOS patients was significantly lower than the control group ($p<0.05$).
Limitations, reasons for caution: The *KISS1, KISS1R, BCL-2, CASPASE-3, FSHR, LHCGR, STAR, ESR1, ESR2, INHBA* gene expression levels of apoptotic process is still under analyses. The protein expression analyses of these genes may strength the results.
Wider implications of the findings: Using kisspeptin antagonists may be supposed as an additional treatment for patients with PCOS.
Trial registration number: OMU KAEK 2019 /724

P-711 The association between thyroid autoimmunity and serum level of anti-Müllerian hormone in infertile patients with normal ovarian reserve undergoing in vitro fertilisation

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Study question: Is there a relationship between thyroid autoimmunity and serum level of anti-müllerian hormon (AMH) in infertile women with normal ovarian reserve undergoing in vitro fertilisation (IVF)?
Summary answer: In infertile women with normal ovarian reserve serum AMH level above 5ng/ml is associated with higher level of thyroid hormones and less frequent thyroid autoimmunity

What is known already: Previous studies suggest that thyroid autoimmunity is associated with a decreased ovarian reserve. Moreover, it was reported that thyroid hormone administration could improve serum AMH level. However, the relationship between serum AMH level and thyroid autoimmunity and function in infertile women with normal ovarian reserve undergoing IVF is largely unknown. Since in IVF the serum AMH level is an important marker which dictate the management of the couple, the identification of all the factors possibly related to this parameter is very important. Study design, size, duration: We performed a retrospective study in the Department of Reproductive Medicine of a private hospital. The medical records of all consecutive patients who underwent IVF between January 2015 and December 2018 with all causes of infertility were reviewed. Study group included 581patients with a mean age of 34.4±4.1 years, mean AMH of 3.78±2.4 ng/mL, mean serum TSH level of 1.89±1 microUI/ml and mean serum free T4 level of 1.05±0.98 ng/dl.
Participants/materials, setting, methods: Patients with known thyroid disorders or under thyroid hormone treatment at the moment of evaluation were excluded. Only patients with serum level of thyroid stimulating hormone (TSH), free tyroxine (free T4), anti thyroid peroxidase antibodies (ATPO,) anti thyroglobulin antibodies (ATG), AMH and age available for analysis were included in the study. This parameters are evaluated on a systematic basis in all the patients undergoing IVF in our Department, except very few cases.
Main results and the role of chance: Patients were divided according to their serum AMH level in two groups: group 1 with AMH level 5 ng/ml and below (n=450 patients) and group 2 with AMH above 5 ng/ml (n=131 patients). When the two groups were compared we found that patients in group 2 were younger in comparison with patients in group 1 (32.9±3.8 versus 35±4 years, p<0.0001). After adjustment for age, patients in group 2 had significantly higher serum free T4 level (1.07±0.12 versus 1.04±0.14 ng/dl, p=0.015), lower ATG (17.59±41.8 UI/ml versus 39.4±136.16 UI/ml, p<0.018) and presented less frequently with high ATPO antibodies (35% versus 41.8%, p=0.047). In a logistic regression model with AMH as a dependent variable, free T4, but not TSH was independently and positively associated with higher AMH levels (above 5 ng/ml) (p=0.025) after adjustment for anti thyroid antibodies levels. Morever, in this logistic model the presence of high ATPO, but not ATG, were negatively related to higher AMH level (p=0.037).
Limitations, reasons for caution: Patients included in this study are infertile patients with indication for IVF treatment. Therefore, the results of this study should be used with caution in other populations
Wider implications of the findings: Our study suggest that serum AMH level might be related to thyroid autoimmunity, but also to thyroid hormones levels. If confirmed by further studies, this findings could offer a way to improve serum AMH level and to better understand the markers of ovarian reserve in an IVF setting.
Trial registration number: NA

P-712 Ovulation induction in type 1 anovulation: a comparative study using gonadotrophins and the GnRH pump

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Study question: Is there a difference in treatment outcome between gonadotrophin releasing hormone (GnRH) pump or hMG for women with Type 1 anovulation undergoing ovulation induction?
Summary answer: Treatment with GnRH was more efficient compared to hMG, with fewer number of cycles to pregnancy, fewer days of stimulation and fewer cycle cancellations.
What is known already: Whilst there is a lot of information on ovulation induction in WHO type II anovulation (PCOS), type 1 anovulation is under-represented in research. WHO type 1 anovulation is characterised by low pituitary gonadotrophins and oestradiol. Treatment options used to include induction of ovulation using gonadotrophins (hMG) or the Gonadotrophin hormone releasing hormone (GnRH) pump delivering pulsatile GnRH. Since the withdrawal of GnRH pump, options have become limited. One study reveals that monofollicular cycles are lower and cycle cancellation higher in women with Type 1 anovulation women treated with gonadotrophins. Study design, size, duration: This is a single centre retrospective cohort study. All women with a diagnosis of WHO type 1 anovulation attending the Reproductive Medicine Unit at the University College London Hospital who received ovulation induction treatment using either hMG or GnRH pump between 1993 and 2020 were included in the study
Participants/materials, setting, methods: 147 women with WHO type 1 anovulation were included in the study. Diagnosis was based on the presence of primary or secondary amenorrhoea in combination with low gonadotrophins and oestradiol. Demographic and clinical data were obtained by reviewing medical records stored within an electronic database. A total of 599 treatment cycles were identified. Statistical analysis between the groups was performed using the independent T test and chi squared test.
Main results and the role of chance: 147 women with WHO type 1 anovulation underwent ovulation induction. hMG was used in 500 cycles (83.5%) and the GnRH pump in 99 cycles (16.5%). Per cycle started the pregnancy rate in the hMG cycles was 107/500 (21.4%) and in the GnRH pump cycles was 19/99 (19.2%) p=0.36. Cycle cancellation was significantly greater in hMG than GnRH pump cycles (hMG 137/ 500 27.4% vs GnRH pump 17/99 17.2% p=0.02). Over response was more common in hMG cycles than GnRH pump cycles (66/130 50.8% vs 3/16 18.8% p=0.01). A total of 363/500 (72.5%) cycles in the hMG and 82/99 (82.8%) cycles in the GnRH pump group reached ovulation. There was no difference in the pregnancy rate after ovulation (hMG 107/363 29.5% vs GnRH pump 19/82 23.2% p=0.15). The mean number of treatment cycles to achieve pregnancy was significantly fewer with the GnRH pump compared to hMG (1.8 (min 1 – max 3) vs 2.4 (min 1 – max 8) p= 0.03).The mean days of stimulation required to reach ovulation was also significantly less with the GnRH pump compared to hMG (16.7 (min 8 – max 34) vs 23.4 (min 7 – max 72) p=<0.001).
Limitations, reasons for caution: This is a retrospective cohort study and is reliant on the quality and quantity of the data entry at the time of clinical treatment.
Wider implications of the findings: Ovulation induction for women with type 1 anovulation is now restricted to a single treatment, namely hMG. hMG is not as effective or optimal as GnRH. Reinstating GnRH in routine clinical practice should be promoted to allow more individualised treatment options and prevent the premature need for in vitro fertilisation..
Trial registration number: NA

P-713 The effect of aging and polycystic ovarian syndrome on the maturation and apoptosis of granulosa cells from women undergoing controlled ovarian hyperstimulation with recombinant hFSH

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Study question: What is the effect of biological aging and polycystic ovarian syndrome (PCOS) on steroidogenic and apoptotic pathways of granulosa cells isolated from women undergoing IVF?
Summary answer: This analysis revealed that aging as well as PCOS influence the response of women to ovarian stimulation, affecting steroidogenesis and apoptosis on granulosa cells.
What is known already: Infertility and subfertility affect a significant proportion of humans worldwide. Age-related factors in women lead to a decrease in the ovarian oocyte reserve, and PCOS, a pathological condition characterized by anovulation and hyperandrogenism, are the two most common reasons of subfertility. Despite the progress in recent years, the causes are still ill-defined, and there is a strong need for interventions to ensure good quality oocytes in healthy aging, as well as in PCOS women. Gene expression analyses of granulosa cells isolated during oocyte retrieval are highly informative for understanding the communication between oocytes and follicular cells and optimizing IVF outcomes.
Study design, size, duration: This report is part of an ongoing study starting from June 2020. 42 women undergoing IVF/ICSI at the University Hospital of

Ioannina, Greece, volunteered to participate with informed consent. Participants were divided into three groups: 30- to 35-year-old patients with PCOS, 30- to 35-year-old non-PCOS women, and older women aged 38- to 42-year-old. All participants received 150 – 300IU/d recombinant hFSH. Participants/materials, setting, methods: Granulosa cells (GC) were collected from the follicular fluid at the day of oocyte retrieval using the protocol described by Ferrero et al., 2012 with modifications that allow us to isolate nearly pure populations, also from small or very few follicles. GC purity was determined by FACS and immunocytochemistry. Reverse Transcription-quantitative-PCR was employed to correlate the expression of steroidogenesis- and apoptosis-related genes with the clinical characteristics of each participant, including FSH and progesterone/estradiol serum levels.

Main results and the role of chance: FACS and immucytochemistry analysis using specific markers of granulosa cells, like FSHR, as well as leucocyte and erythrocyte cell markers (CD45 and Glycophorin) confirmed the purity of the isolated granulosa cell populations. No differences were found in hormone serum levels between 30- to 35-year-old PCOS (Group A) and non-PCOS women of the same age (Group B) whereas progesterone and estradiol serum levels were lower in 38- to 42-year-old women (Group C) compared to group B. RT-qPCR analysis of maturation-related genes (*ZP3* and *AHR*) revealed the presence of more immature follicles in Group A compared to the other groups. The granulosa cells from women of group A were characterized by increased expression of genes involved in steroidogenic pathways (such as *FSHR*, *CYP17A1*) compared to group B, as well as of the genes for the estradiol and progesterone receptors (*ESR1* and *PGRMC1*, respectively), and by decreased levels of the apoptosis-related genes *BAX* and *BCL-xL*. Importantly, the granulosa cells from the women of Group C were characterized by lower mRNA levels of steroidogenesis-related genes, as well as of *ESR1*, and by increased apoptosis as revealed by the increased ratio of *BAX/BCL2* transcripts, compared to Group A.

Limitations, reasons for caution: The presented results of gene expression analysis are based on preliminary data as the number of participants is small (6 women per group), but they are part of an ongoing study where the number of participants is increasing in order to improve quantification and reproducibility of our results.

Wider implications of the findings: Potential differences in signaling pathways that are crucial for the maturation of granulosa cells and follicles during biological aging or in pathological conditions such as PCOS are expected to provide valuable information concerning the response of individual women to gonadotropin stimulation and might help to design more personalized therapeutic strategies.

Trial registration number: not applicable

P-714 Insulin-like growth factor-I as a mediator of the effect of transdermal testosterone in poor responder patients

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Study question: Is insulin-like growth factor-I (IGF-I) a mediator of the effect of transdermal testosterone (TT) in poor responder (PR) patients?

Summary answer: IGF-I might be a mediator of the effect of TT in PR patients who undergo an IVF cycle

What is known already: Many strategies have been tried to improve the results in PR patients. Androgen supplementation with TT is the only that has significantly increased live birth rate in these patients. The mechanism by which TT might influence on the better results remains unclear but it is likely mediated or facilitated by IGF-I. Testosterone increases the number of primordial follicles, increase IGF-I by threefold and increase IGF-I receptor mRNA by fivehold in primordial follicles in primates. Some studies have suggested that IGF-I could be a parameter that reflects the endocrinological environment of mature follicles, which is correlated with oocyte and embryonic quality

Study design, size, duration: This prospective cohort study of 93 women PR according Bologna criteria treated with TT and IVF/ICSI was conducted between May 2015 and December 2016

Participants/materials, setting, methods: Exogenous andogenization with TT for 5 days prior to ovarian stimulation was carried out. Hormonal parameters were evaluated: basal FSH, LH and Estradiol, AMH, IGF-1 pre and post TT. Ultrasound parameterswere also analysed: antral follicle count (AFC) and number of pre-ovulatory follicles the day of HCGr.

We compared these parameters according to the ovarian response: adequate (> 4 oocytes) or insufficient (<3 oocytes), as well as the pregnancy was achieved or not.

Main results and the role of chance: Baseline characteristics of the patients were: 36.9 years, FSH 11.8, AMH 0.86 and RFA 5.3. In 83% of the patients the oocyte retrieval was carried out, obtaining an average of 3.8 MII oocytes and 2.9 embryos of 2pn with a clinical pregnancy rate per transfer of 33.3%. The FORT Test (AFC/pre-ovulatory follicles x100) was 70%, higher than that observed in other studies with patients with PR without TT (55%).

In cases in which an insufficient response was obtained (<3 oocytes) or the cycle was canceled, a higher age and FSH and lower AMH were observed ($p < 0.05$). There were no differences in the rest of the parameters.

Evaluating the hormonal and ultrasound parameters depending on whether or not pregnancy was achieved, a significant increase in IGF1 pre and post-TT was observed in the cases of pregnancy (31.5%) compared to those cases where there was no pregnancy (10.9 %) (p=0'01). There were no differences in the rest of the parameters.

A significant correlation was found between AMH, AFC and increase in IGF-I levels (p<0'05).

Limitations, reasons for caution: This a prospective cohort study with limited number of patients included.

Wider implications of the findings: The significant increase in serum levels of IGF-1 in pregnant patients would indicate the existence of a more favorable clinical setting for the administration of testosterone, probably related to a more favorable ovarian reserve as demonstrated by its correlation with serum levels of AMH and with the AF.

Trial registration number: not applicable

P-715 Nomogram for anti-Mullerian hormone (AMH) and follicle-stimulating hormone (FSH) in healthy infertile women according to the cause of subfertility

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Study question: Does the cause of subfertility affect any age-related changes observed in anti-Mullerian hormone (AMH) and follicle-stimulating hormone (FSH)?

Summary answer: There was no significant effect of the cause of subfertility on AMH and FSH, however women with unexplained subfertility were significantly older than controls.

What is known already: Ovarian reserve and consequently female fecundity irreversibly diminish with age. Subfertility is an increasingly prevalent clinical presentation with a multitude of underlying pathologies. Ovarian reserve testing, including biomarkers, plays a crucial role in the management of subfertility, particularly in predicting ovarian response during in-vitro fertilisation (IVF) treatment. For some couples, no cause can be identified and their subfertility remains unexplained thus making prognosis and treatment challenging. It has been proposed that these couples may have an ovarian reserve at the extreme lower end of normal. Study design, size, duration: This clinical audit retrospectively investigated 864 subfertile healthy women who had undergone investigation at the Royal Derby Hospital fertility clinic. Data were collected in Excel including age, serum AMH, serum FSH if available, and subfertility diagnosis. The data were collected from a pre-existing database produced by a group of researchers pre-2016 at the Royal Derby Hospital. The researchers had access to pathology lab reports and computerised hospital records containing clinical details. Participants/materials, setting, methods: Subfertility diagnoses were categorised by cause: male factor and tubal factor were combined and served as a control group, oligo-anovulatory factor, endometriosis factor, and unexplained subfertility. If multiple factors were present, oligo-anovulation was taken as the presiding factor. One-way ANOVA using Minitab was used for statistical analysis to assess the effect of cause of subfertility on age, on AMH, and on FSH. For AMH and FSH, age was incorporated as a covariate.

Main results and the role of chance: AMH significantly decreased with age ($p < 0.001$) and FSH significantly increased with age ($p < 0.05$). The age-related change in AMH was more pronounced than in FSH. The cause of subfertility had a statistically significant effect on age. The nature of this was that the unexplained group was significantly older than the control group and the

oligo-anovulatory group (p<0.001). Compared to the control group, AMH was lower in the unexplained and endometriosis groups and higher in the oligo-anovulatory group. However, after adjusting for age, the effect of cause of subfertility on AMH was not significant (R2(adj)=24.91%, p>0.05). Compared to the control group, FSH was lower in the unexplained, oligo-anovulatory, and endometriosis groups. However, after adjusting for age, the effect of cause of subfertility on FSH was not significant (R2(adj)=2.05%), p>0.05).
Limitations, reasons for caution: The previous group of researchers may have exhibited selection bias and clinical interpretation during data collection. The study population was unevenly distributed across the different causes of subfertility. Only the effect of age was accounted for despite many factors being known to affect female fertility.
Wider implications of the findings: Respective nomograms for AMH and FSH according to cause of subfertility provide a reference point for clinicians, especially to predict ovarian response during IVF treatment. Although AMH was not significantly lower in the unexplained group compared to the control group, the women were significantly older implying a lower ovarian reserve.
Trial registration number: not applicable

POSTER VIEWING
REPRODUCTIVE EPIDEMIOLOGY, SOCIO-CULTURAL ASPECTS AND HEALTH ECONOMY

P-716 The relationship of men's adherence to the Mediterranean diet with sperm parameters and outcomes of assisted reproductive technologies

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Study question: Is men's adherence to the Mediterranean diet (MD) associated with sperm parameters and couples' outcomes of assisted reproductive technologies (ART)?
Summary answer: Higher men's adherence to the MD was associated with an increased probability of clinical pregnancy and live birth among couples undergoing ART.
What is known already: There is growing literature supporting the hypothesis that some nutrients, foods, and dietary patterns may be related to sperm quality and fertility. However, no previous studies analyzed the relationship of men's adherence to the Mediterranean diet with sperm parameters and ART outcomes in the same cohort.
Study design, size, duration: The Environmental and Reproductive Health (EARTH) Study is a prospective preconception cohort of couples seeking fertility treatment at the Massachusetts General Hospital Fertility Center (Boston, USA). This analysis includes 314 men and their female partner, who underwent 791 ART cycles (2007-2020). Diet intake was measured by a validated semi-quantitative food frequency questionnaire completed prior to ART. Men's adherence to the MD was estimated using the Trichopoulou score.
Participants/materials, setting, methods: Primary outcomes included: conventional sperm parameters (volume, sperm count, concentration, motility, and morphology), and ART outcomes (implantation, clinical pregnancy, and live birth). We estimated the marginal means and 95% confidence interval (95%CI) for semen parameters and the probability of ART (95%CI) by employing generalized linear mixed models while adjusting for several potential confounders. Sensitivity analyses by changing the cut-off points of adherence to the MD were tested.
Main results and the role of chance: At baseline, men had a median (IQR) age of 35.6 (32.6, 38.8) years and a BMI of 26.7 (24.0, 29.4) kg/m2. Female partner age median (IQR) was 35.0 years (32.0, 38.0) and BMI 23.3 (21.3, 26.6) kg/m2. Couples were mostly white and had never smoked. Men's adherence to the MD was not associated with seminal parameters in the multivariable-adjusted models but it was related to a higher probability of clinical pregnancy and live birth. The predicted marginal proportions and confidence intervals among men in the lowest compared with the highest quartile of adherence to the MD were 0.25 (0.14, 0.40), 0.55 (0.41, 0.68) for clinical pregnancy (P-trend=0.005), and 0.19 (0.10, 0.32), 0.42 (0.30, 0.55) for live birth (P-trend=0.014). Male partner MD dietary pattern scores were unrelated to the probability of implantation. Sensitivity analyses using tertiles and quintiles of men's adherence to the MD showed similar associations.
Limitations, reasons for caution: Although we have adjusted our models by several potential confounding factors, residual confounding cannot be ruled out, and therefore our results should be interpreted with caution. The findings may not be generalizable to other populations or couples attempting conception without ART.
Wider implications of the findings: According to our knowledge, this is the first study suggesting that adherence to MD could be suitable dietary guidance for men whose partners are undergoing infertility treatment.
Trial registration number: Not applicable

P-717 Where do cryopreserved embryos end up after a positive pregnancy test?

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Study question: What is the destination of supernumerary embryos after a positive pregnancy test?
Summary answer: Half of the surplus cryopreserved embryos in assisted reproduction treatments are not transferred.
What is known already: Many of the surpernumerary cryopreserved embryos in assisted reproductive technologies are not transferred. This is a constant issue in many fertility centers around the world. Our objective was to report what happens with vitried surplus embryos after IVF in patients with a positive pregnancy test, carrying out an analysis according to age and final evolution of the pregnancy.
Study design, size, duration: This is a retrospective descriptive study. We analyzed 245 embryo transfer cycles, performed between January 2013 to December 2017, in 235 patients with a positive pregnancy test and who vitrified surplus embryos.
Participants/materials, setting, methods: All the patients underwent treatment with their own oocytes. The variables studied were: age, miscarriage rate (MR) and live birth rate (LBR). We compared the destination of the cryopreserved embryos according to the patient's age and pregnancy evolution. Statistical analysis was performed with Fisher's exact test.
Main results and the role of chance: 20% of the IVF cycles (n=49) were performed in women older than 40 years, 42% between 35 and 39 (n =103) and 38% in women younger than 35 (n= 94). Average age was 35.8 ± 4.1 years. 859 embryos were cryopreserved (3.5 ± 1.9 cryopreserved embryos/patient). Average search time for surplus embryos was 20.5 ± 17.9 months, rising to 36.9 ± 14.9 months after delivery and decreasing to 8.7 ± 7.8 months after miscarriage (P<0.0001). Up to date there are 118 (48.2%) patients whose cryopreserved embryos have not been transferred yet. Signficant differences were found in the three groups in using the cryopreserved embryos according to whether or not they had delivery. Almost half of the surplus cryopreserved embryos are not transferred. Regardless of the age of the patient, all groups showed the same behavior regarding the utilization of the cryopreserved embryos after delivery. It is essential to advise couples who perform assisted reproductive technologies, with a good probability of success (regardless of the patient's age), about the responsibility that embryonic cryopreservation entails. Argentine legislation has limitations regarding the availability of cryopreserved surplus embryos.
Limitations, reasons for caution: This is a retrospective study.
Wider implications of the findings: We believe that Public Health policies related to this issue should be re evaluated based on these results.
Trial registration number: not applicable

P-718 Paternal smoking in the preconception period is associated with an increased risk of spontaneous miscarriage in a dose-dependent manner: a systematic review and meta-analysis

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Study question: What is the association between paternal lifestyle factors in the preconception period and the risk of spontaneous miscarriage? Summary answer: Preconception paternal cigarette smoking is associated with an increased risk of spontaneous miscarriage, while no associations were found with paternal alcohol consumption and obesity.
What is known already: Although maternal lifestyle risk factors for miscarriage are well-established, studies on potentially contributing paternal factors remain sparse. Recently, a significant association was found between advanced paternal age and spontaneous miscarriage. Biological evidence indicates that smoking, excessive alcohol consumption and obesity may lead to sperm oxidative DNA damage, being a known risk factor for miscarriage. Study design, size, duration: Systematic review and meta-analysis.
Participants/materials, setting, methods: PubMed and Embase databases were searched in August 2020. Paternal factors examined were: cigarette smoking, alcohol consumption and Body Mass Index (BMI). A qualitative risk of bias assessment was performed for all included studies. Meta-analysis was performed if sufficient data was available from studies that controlled for maternal factors. PRISMA guidelines for systematic reviews were followed.
Main results and the role of chance: The systematic search included 3386 articles of which 11 articles met the inclusion criteria. In a meta-analysis of eight studies, paternal smoking of >10 cigarettes per day in the preconception period was found to be associated with an increased risk of spontaneous miscarriage, after adjustment for maternal smoking status (1-10 cigarettes per day: 1.01, 95% CI 0.97-1.06; 11-20 cigarettes per day: 1.12, 95% CI 1.08-1.16; >20 cigarettes per day: 1.23, 95% CI 1.17-1.29). Based on five available studies, no clear association was found between paternal alcohol consumption and spontaneous miscarriage. No studies were retrieved that evaluated the association between paternal BMI and spontaneous miscarriage.
Limitations, reasons for caution: Investigating the relation between paternal lifestyle factors and spontaneous miscarriage is challenging and prone to different forms of bias, especially in retrospective studies.
Wider implications of the findings: Awareness of the association between heavy paternal smoking in the preconception period and the risk of spontaneous miscarriage should be raised. More well-designed studies are needed to further investigate the effects of other paternal lifestyle factors on the risk of spontaneous miscarriage.
Trial registration number: not applicable

P-719 Self-declared infertility and child desire among women of reproductive age in the National Survey of Demography and Health, Brazil

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Study question: This article aims to characterize from a socio-demographic point of view, women of reproductive age who wish to have children, declared themselves infertile, and their search for treatments and outcomes.
Summary answer: It is essential to develop specific population surveys on infertility in Brazil to identify its magnitude and main economic and social components.
What is known already: Commonly neglected in developing countries where public policy is incipient, infertility brings social, economic and psychological consequences to couples. It is considered as a serious public health problem whose impact varies among different populations and acquires relevance for specific communities. In Brazil, there are no clinical or demographic data that point us to the magnitude of the problem, its social characteristics and impact. Taking into account the postponement of motherhood for after 30 years, there will probably be an increase in the number of women and couples who may resort to infertility treatments to fulfil the desire for procreation.
Study design, size, duration: The National Survey of Demography and Health of Women and Children (PNDS) is a cross-sectional study and a household complex probabilistic sampling. The sampling units were selected according to a stratified model of simple random conglomerates in two stages: lottery draw and household draw. The last survey was conducted between June 2006 and May 2007 in 14,617 households. In the selected households, interviews were conducted with 15,575 women of reproductive age.
Participants/materials, setting, methods: The participants consisted of 15.575 women between 15 and 49 years, representative of the five Brazilian macro-regions. The information was obtained through questionnaires, applied in person, raising information on fertility, fecundity, contraception, use of health services and socioeconomic profile. The interviewer's team was formed by approximately 100 people and 27 supervisors, all-female, divided into nine regional teams. The system used for data entry was the Census and Survey Processing System - CSPro.
Main results and the role of chance: The survey results indicate that of women who wish to have children, 9.2% declared themselves infertile; 50,8% of them sought health services for treatment; non-black women had higher percentages of demand compared to black women (62.4% versus 41.3%). Also, there were higher percentages of seeking help from women belonging to classes A (61.2%), B (83.3%) and C (60.9%) compared to those belonging to classes D (30.4%) and E (7.8%) On the other side, almost half of women did not seek help to get pregnant (49,1%); this percentage is higher among black women (58%). Moreover, women in classes D and E had the highest percentages of non-demand, 69.6% and 92.2%, respectively. The reasons cited for those who do not seek help, are "I think there is no solution" (54,7%); "I don't think I can get help" (17.3%), "financial reasons" (26.8%) or "I don't know where to get it" (1,2%). Among those who sought help, 48,5% are under treatment, 24,4 % said there is no solution; 15,8% are waiting for service and 11,3% have no money for treatment. Significance limit was established for values of $p < 0.05$. The analysis was performed in the programs Stata v.9 and/or SPSS v.14.
Limitations, reasons for caution: The limitations of the study are recognized. Firstly, opinions are restricted to the moment of the interview and, thus, the desire for children may change over time. Secondly, the statement of infertility is based on self-declaration, not on clinical diagnosis.
Wider implications of the findings: This is the first study based on PNDS 2006 data on infertility and demand for treatments in Brazil. It can contribute to providing insights, raising new questions and discovering relevant categories and dimensions of analysis to be taken into account in future studies and surveys.
Trial registration number: not applicable

P-720 Prevalence of Female Infertility in the UK Armed Forces

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Study question: What is the prevalence of female infertility among UK military personnel and does it differ from the Metropolitan Police Service (MPS) and civilian populations?
Summary answer: Prevalence of self-reported infertility was higher in servicewomen (31.7%) and female MPS officers (36.3%) than in civilian women (24.4%).
What is known already: Arduous employment is associated with numerous potential occupational hazards and behaviours that may be relevant to fertility. These include physical and psychological stress, smoking, alcohol drinking and other lifestyle factors. A preliminarily report in 2016 indicated that UK

servicewomen over 30 years of age were more likely to present with fertility problems compared with reported civilian infertility data for age-matched women. Few previous studies have compared infertility prevalence of servicewomen with civilians, and none have compared infertility prevalence with other occupations.

Study design, size, duration: A cross-sectional study was undertaken in 2019 to determine prevalence of infertility. All eligible UK servicewomen (14,650) and MPS officers (8,262) aged 18-60 years were invited to participate with sisters of participants recruited as controls using a snowball technique. Data including pregnancy history, time to each pregnancy and self-reported infertility risk-factors were collected using an online questionnaire. We estimated a sample of 4898 servicewomen would give a precision of 1% around infertility prevalence estimates.

Participants/materials, setting, methods: The questionnaire was developed, piloted and adapted for electronic distribution. The occupational groups were invited by email to complete the questionnaire on three occasions. Prevalence of infertility was defined as the proportion of women at risk of pregnancy who had not become pregnant within 12 months. Only women with pregnancy outcomes, or fully tested for fertility (12 months or more of exposure), were included in the denominator.

Main results and the role of chance: Participants included 4806 (33%) women serving in the UK Armed Forces, 1237 (15%) female MPS officers and 435 (estimated 8%) non-military, non-MPS sisters (biological, half, step or adopted) of both groups. 98.4% of responses were complete. Prevalence of self-reported 12-month infertility was 31.7% (95% CI 29.9-33.5) in servicewomen, 36.3% (95% CI 33.1-39.7) in MPS officers and 24.4% (95% CI 19.6-29.8) in civilian women. Age, history of polycystic ovary syndrome, endometriosis, fibroids, tubal and pelvic surgery, hysterectomy and a short General Health Questionnaire (GHQ 12) score of >4 (suggesting a minor psychiatric disorder) were associated with infertility and adjusted for in logistic regression models to estimate odds ratios. The adjusted odds ratio (aOR) of infertility in servicewomen was 1.0 (95% CI 0.8-1.2) compared with MPS officers and 1.5 (95% CI 1.1-2.0) in both servicewomen and MPS officers compared with sisters.

Limitations, reasons for caution: The major limitation is the low response rate, particularly in the two control groups, potentially resulting in response bias. Prevalence of infertility could have been further over-estimated if fertile women are more likely to have left the military or MPS. There is scope for residual confounding.

Wider implications of the findings: Further analyses will explore the key risk factors to identify what aspects of these occupations contribute to infertility and which may be modifiable. Future cohort studies would be helpful to extend the understanding of the influence of occupation on infertility.

Trial registration number: not applicable

P-721 Probability of receiving assisted reproductive technology treatment through out-of-pocket payment and household income: A discrete choice experiment in Japan

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Study question: What is the probability that patients will receive assisted reproductive technology (ART) treatment based on their out-of-pocket payment and income class?

Summary answer: Higher-income patients opted for ART even at a higher cost, whereas an out-of-pocket payment was the most influential determinant in all income groups.

What is known already: Economic disparities affect access to ART treatment in many countries. At the time of this survey, Japan provided partial reimbursement for ART treatment exclusively for those in low- or middle-income classes due to limited governmental budgets. However, the optimal financial support by income class is unknown.

Study design, size, duration: We conducted a discrete choice experiment (DCE) in Japan in January 2020 including 824 women with fertility problems who were recruited via an online social research panel.

Participants/materials, setting, methods: Participants included women aged 25–44 years undergoing fertility diagnosis or treatment. They completed a DCE questionnaire including 16 hypothetical scenarios, created by orthogonal design, to measure six relevant ART attributes (pregnancy rate, risk of adverse effects, number of visits to outpatient clinics, consultation hours, kindness of staff, and out-of-pocket expense) and their relation to treatment choice. We used mixed-effect logistic regression models to estimate the probability of receiving ART treatment for each attribute.

Main results and the role of chance: Of the 1,247 eligible women recruited, 824 completed the survey (66% participation rate). All six attributes significantly influenced treatment preference, with participants valuing out-of-pocket payment the most, followed by pregnancy rates and kindness of staff. The odds ratios of each attribute to receiving ART treatment were 0.58 (95% confidence interval [CI]: 0.57−0.59) for out-of-pocket payments per additional 100,000 Japanese yen (JPY; i.e., 800 euros), 1.47 (95% CI: 1.43−1.53) for pregnancy rates per additional 5%, and 4.16 (95% CI: 3.73−4.64) for kindness of staff, after adjusting for clinical and socioeconomic factors. Significant interactions occurred between high household income (≥8 million JPY) and high out-of-pocket payment (≥500,000 JPY). However, the mean predicted probability of the highest-income patients (i.e., ≥10 million JPY) to receive ART treatment at the average cost without public funding (i.e., 400,000 JPY) was 47% (interquartile range: 18%−76%), whereas that of middle-income patients (i.e., 6–8 million JPY) to receive ART at the average subsidized cost (i.e., 100,000 JPY) was 60% (interquartile range: 33%–88%).

Limitations, reasons for caution: Other attributes not included in our DCE scenarios might be relevant in real-life settings. Choices made in a DCE would not wholly match the actual treatment choices.

Wider implications of the findings: The present DCE suggested that out-of-pocket payment was the primary determinant in patients' ART decisions. High-income patients were more likely to receive ART treatment even at a high cost, but their ineligibility for government financial support due to their high income might discourage them from receiving treatment.

Trial registration number: NA

P-722 Role of medical practices in early drop-out in infertility care

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Study question: What is the burden of early infertility care drop-out in the context of full reimbursement of infertility care and are medical practices associated with drop-out?

Summary answer: Medical practices seem associated with early infertility care drop-out whose burden remains high despite the equality of care policy in France

What is known already: More than half of couples drop out of their infertility treatment before pregnancy. Direct costs of infertility care are widely cited as the major barrier to continuation of care. In France, infertility treatments are fully reimbursed. Data from US IVF centres have shown a high estimated drop-out from the beginning and at each step of infertility treatment. Some studies have suggested a difference in medical practices during infertility care between couples who have dropped out and couples who have continued care

Study design, size, duration: Cohort study of all women aged 18–49 years who started infertility treatment between January–August 2016 in mainland France with a private practitioner and who had not been successfully treated at a 24-month follow-up.
Participants/materials, setting, methods: The cohort was based on the French national health insurance and hospital databases that exhaustively record reimbursed healthcare. Infertility treatment included ovarian induction by gonadotropin and clomifene. Outcome was infertility care drop-out within 3 months after starting treatment. Stratified analyses were performed based on the first treatment prescribed, with three categories: clomifene prescribed by a general practitioner, clomifene by a gynaecologist or gonadotropin by a gynaecologist
Main results and the role of chance: Among women unsuccessfully treated by ovarian induction, 31.1% dropped out of infertility care within 3 months. Women who started treatment with a general practitioner were more frequently socially disadvantaged (40.6%), less closely monitored (ultrasound and hormonal tests had not been done in 53.6% and 64.8%, respectively) and dropped out more frequently (46.8%). Where the prescriber of treatment was a gynaecologist, older age (35-39 and 40-43 years) was associated with higher probability of early drop-out with OR 1.5 (95% CI 1.3-1.7) and 2.2 (95% CI 1.9-2.5) for gonadotropins and 1.2 (95% CI 1.1-1.3) and 1.9 (95% CI 1.7-2.2) for clomifene. Disadvantaged social status was associated with higher early infertility care drop-out, whereas good monitoring was associated with a lower probability of early drop-out in all three categories of treatments and prescribers
Limitations, reasons for caution: These medical and administrative data did not allow us to explore medical practices in greater depth, notably the doctor-patient relationship
Wider implications of the findings: This is the first estimation of early infertility care drop-out in a general population where treatment is fully reimbursed, exploring the implication of medical practices in early drop-out. Better access to good medical practices is needed for less socially advantaged people, beyond the current equality of care policy in France.
Trial registration number: N/A

P-723 Ovarian reserve parameters and ovarian stimulation outcome for IVF/ICSI are influenced by ethnicity

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Study question: Are the ovarian reserve parameters and the outcome of ovarian stimulation for IVF / ICSI influenced by ethnicity?
Summary answer: Ethnicity influences ovarian reserve parameters and the outcome of ovarian stimulation for IVF / ICSI
What is known already: Infertility affects couples worldwide and due to a lack of a standardized reporting system, the real number, especially in developing countries, might be underestimated. The etiology of infertility may differ around the world and is often subjected not only to social, cultural and religious peculiarities, but also to different genetic influences. Published data suggest that ethnicity influences the ovarian reserve as well as the outcome of Assisted-Reproductive-Techniques (ART)-treatments. Key players of a successful ART outcome are the ovarian reserve and consequently the number of oocytes retrieved. Until today, the impact of ethnical differences is not sufficiently addressed in research.
Study design, size, duration: Prospective observational study, performed in 10 infertility centers worldwide (Europe (4 centers), Middle East North Africa (MENA) region (2 centers), Iran (2 centers), South America (1 center), India (1 center)) between May 2019 and September 2020, evaluating ovarian reserve and outcome parameters of ovarian stimulation treatments for IVF/ICSI. The study was approved by the Ethical Committee of each participating center.
Participants/materials, setting, methods: Couples with primary / secondary infertility and an indication for an IVF/ICSI treatment were included into this study. Besides anamnestic data regarding the history of the infertility and self-reported ethnicity (Arab, Caucasian, Hispanic, Ohters, Persian and South Asian), data obtained during the basic fertility assessment on the ovarian reserve parameters (Antral follicle count (AFC) and Anti-Muellerian-Hormone (AMH)) as well as stimulation parameters from the ovarian stimulation treatment were collected and analyzed.
Main results and the role of chance: This study comprised 1032 couples with the following distribution of the ethnicities: Arab 21.5%, Caucasian 15.9%, Hispanic 5%, Others 1.2%, Persian 33.4%, and South Asian 23%. The unadjusted means, SD and 95%CI (Confidence Interval) of AMH (ng/ml) for the groups were 3.33±0.19 [2.95-3.71]; 2.03±0.25 [1.55-2.52]; 2.43±0.74 [0.97-3.88]; 2.76±0.96 [0.88-4.64]; 3.10±0.16 [2.79-3.41]; 3.62±0.19 [3.25-3.98], for AFC 15.52±0.53 [14.49-16.55]; 12.00±0.67 [10.69-13.31]; 12.69±1.08 [10.57-14.81]; 15.11±2.60 [10.01-20.21]; 13.58±0.42 [12.75-14.41]; 13.49±0.51 [12.49-14.48] and for the number of retrieved oocytes (rCOC) 14.08±0.61 [12.88-15.27]; 9.84±0.71 [8.44-11.24]; 7.94±1.26 [5.48-10.41]; 9.92±2.62 [4.78-15.05]; 10.83±0.49 [9.87-11.79]; 17.06±0.59 [15.90-18.21], respectively. Univariate analysis of AMH, AFC and rCOC with the ethnicities revealed highly statistically significant differences for AMH and rCOC ($p < 0.001$, respectively) and significant differences for AFC ($p = 0.0014$).

As age is a major confounder for the ovarian reserve, multivariate analyses were performed. After adjusting for age, AMH was significantly different between Arab-Persian, Arab-South Asian and Arab-Caucasian ($p < 0.001$, $p < 0.001$, $p = 0.002$) and AFC statistically significant between Arab and all other ethnicities. For rCOC, besides age, also the stimulation-dosage and -duration was taken into account. Highly statistically significant differences were found for the groups Arab-Persian and Arab-Caucasian and no differences towards the other ethnical groups.
Limitations, reasons for caution: Limitations of the study are an unequal number of included patients per ethnicity and that the data for the ovarian reserve parameters and the stimulation outcome were not available for all of the included patients.
Wider implications of the findings: Counselling of couples with infertility have to take, besides all other factors, also the ethnicity of the couple into account as ethnicity influences the ovarian reserve parameters as well as the number of retrieved oocytes in ovarian stimulation cycles for IVF/ICSI.
Trial registration number: ClinicalTrials.gov Identifier: NCT03927417

P-724 The association between use of assisted reproductive techniques and childhood asthma: a Swedish nationwide register-based cohort study

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Study question: Are the previously reported greater risks of childhood asthma in children conceived by assisted reproductive techniques due to the intervention or unmeasured parental confounding?
Summary answer: After accounting for both measured and unmeasured parental factors we found no indication that the use of assisted reproductive techniques increases children's risk of asthma.
What is known already: Several earlier studies have reported a higher risk of childhood asthma among children conceived by ART. However, only one previous study has attempted a sibling comparison to account for infertility as well as parental background factors, and their findings need to be replicated. Little is thus known on what underlies the higher risk of childhood asthma.
Study design, size, duration: In this nationwide register-based cohort, we identified all 1,671,532 live births between 1997 and 2013 in the Swedish Medical Birth Register (MBR) and followed them to the end of 2018.

Participants/materials, setting, methods: Infertility and ART use were ascertained from IVF clinic reporting, clinical diagnosis, and maternal self-report during the first antenatal visit. Childhood asthma was identified from diagnosis in hospitalization and outpatient specialist care records, and dispensations of asthma medication. Cox proportional hazard regression was used to estimate the association of ART and asthma in the population, in children of couples with known infertility, and in a sample of siblings conceived with and without ART (differentially exposed).
Main results and the role of chance: Of the 1,671,532 live births in the cohort, 11.7% were born to couples with known infertility, and 3.5% were conceived with ART. Compared with all other children, children conceived by ART had a small, elevated risk of asthma (adjusted hazard ratio (aHR)=1.14, 95% Confidence interval (CI) 1.11 to 1.16). When the comparison was restricted to children of couples with known infertility the difference in risk was even smaller (aHR=1.07, 95% CI 1.05 to 1.10), and in the comparison of siblings conceived with and without ART no difference in risk was seen (aHR=0.98, 95% CI 0.86 to 1.13). Among children conceived with ART, those in which intra-cytoplasmic sperm injection (ICSI) had been used had a slightly lower risk of asthma (aHR=0.93, 95% CI 0.90 to 0.97), and no difference in risk was seen between use of fresh and frozen-thawed embryo transfer.
Limitations, reasons for caution: Sibling comparison is sensitive to potential misclassification, unmeasured confounding and carryover effects, so should be interpreted with this in mind. Differences in treatment implementation across time and settings could affect the ability to extrapolate the conclusions to another clinical context (where e.g., single-embryo transfer policy is not implemented).
Wider implications of the findings: This study found a modestly elevated risk of asthma in children conceived with ART to be largely explained by confounding from parental background factors. There were further no indications of adverse influence from increasingly utilized ART procedures such as ICSI or embryo-freezing, with respect to asthma in childhood.
Trial registration number: not applicable

P-725 COVID-19: Fertility patients' attitudes to pregnancy and implemented changes within fertility services during a pandemic

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Study question: To investigate the attitudes of male and female fertility patients to risk mitigation strategies and pregnancy advice during the first wave of the COVID-19 pandemic.
Summary answer: The desire to conceive outweighed fears regarding infection. Patients felt fertility treatments should be classified as essential and were agreeable to most risk mitigation strategies.
What is known already: The effects of the COVID-19 global pandemic on fertility services became evident in early 2020. Because of possible impacts of the virus on gametes, embryos and patients and concerns over virus transmission and the ability of medical services to cope, fertility treatments were temporarily suspended, as advised by ESHRE, ASRM and others. Across Europe, services were paused for approximately 7 weeks. Patients have reported that they found this extremely stressful and in some cases, unfair. After the initial closures, many clinics re-opened but with new risk mitigation strategies regarding PPE, hygiene and reducing staff and patient footfall.
Study design, size, duration: Men and women with a scheduled appointment at a fertility clinic over a 7-week period during the first wave of the COVID-19 pandemic were asked to complete a questionnaire outlining their experience and how it affected them. Participants were recruited via email using a secure online patient portal. A standardised anonymous 25-item questionnaire was sent to 828 patients and a reminder was circulated seven days later. The questionnaire remained open for 28 days.
Participants/materials, setting, methods: Participants were invited to complete a questionnaire and assured that all data would remain anonymous. Three areas were assessed. Firstly, how the pandemic itself affected their attempts to conceive. Secondly, participant perceptions regarding the overall disruption to fertility services. Thirdly, how participants feel fertility services should be treated in the event of a future large scale global pandemic.
Main results and the role of chance: 135 responses were received, giving a response rate of 16.3%. 80% of respondents were female and 20% male with no significant difference in responses between the sexes. Most participants (96%) had completed third level education and 90% were fully employed. Interestingly, 69% of participants continued trying to conceive during this time. This was despite 28% having concerns about contracting COVID-19 should they attend a clinic, 21% having concerns regarding the effect of the virus on pregnancy and 21% having concerns regarding an impact on the fetus. The majority surveyed (93%) stated that fertility treatment should be considered essential. 90% had their treatment disrupted or altered and, of these, 44% felt that this was justified, 23% disagreed and 33% were unsure. Regarding changes implemented within the clinic, 68% were satisfied with online video consultations and a further 16% would be content in certain circumstances. 92% felt privacy was maintained and 95% were happy to sign consent forms via video link. Many disagreed with the no partner policy at early pregnancy scans(57%) and embryo transfer(44%); however, they agreed with it for phlebotomy and treatment scans. In the event of a future pandemic, 79% felt fertility services should or probably should be continued.
Limitations, reasons for caution: This study focuses on the first wave of COVID-19. The long term, ongoing nature of the pandemic may influence participants' perspectives on the areas investigated over time.
Wider implications of the findings: It is estimated that the world will face a global pandemic approximately once every generation. Fertility stakeholders must learn from these events and studies such as ours are important to ascertain the views of service users. Some policies, such as video consultations, may be of benefit even in non-pandemic times.
Trial registration number: not applicable

P-726 Analysis of the results of conjugal artificial insemination in a third-level public health hospital

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Study question: Our objective was to compare the results and costs between conjugal intrauterine insemination (IAC) and in vitro fertilization (IVF) in a third-level public health hospital.
Summary answer: The direct estimated cost for achieving a clinical pregnancy was € 3808.24 for IAC and € 14,195.88 for IVF.
What is known already: There is a belief among patients, professionals, and media in favor of the results offered by IVF. Studies on the results, risks, complications and costs are difficult to understand, thus making IVF the most widespread and in demand reproduction technique among fertility clinics. In 2013 the National Institute of Health and Care Excellence conducted a study comparing artificial insemination using 25 mg of clomiphene citrate vs. expectant management of sterility, without finding significant differences. After this, in their guide they recommended the elimination of insemination from the couples' treatment protocols, proposing to replace it with three cycles of IVF.
Study design, size, duration: This is a retrospective observational study of a total of 1384 conjugal insemination cycles carried out in our center between 2007-2019 and 646 cycles of in vitro fertilization, intracytoplasmic injection (ICSI) or mixed.
Participants/materials, setting, methods: All IAC were included. IVF cycles analyzed were those made under the diagnose of tubal factor, to homogenize the samples. The calculation of the direct costs of each technique has been carried out by the collection of the costs of these procedures from the registry of public costs of the Andalusian public health system. The pharmacy spending in an average cycle has been obtained from the public prices of prescriptions made in our service.
Main results and the role of chance: The direct estimated cost for achieving a clinical pregnancy with the cumulative clinical pregnancy rate found in our sample was € 3808.24 for IAC and € 14,195.88 for IVF. The additional cost of a pregnancy achieved by IVF compared to one achieved by IAC was € 10,387.64. The mean age of the patients was higher in the group undergoing in vitro fertilization treatment (34.41 years) compared to those undergoing artificial insemination treatment (32.18 years), the differences between both being statistically

significant (p <0.005). We also found statistically significant differences between the clinical pregnancy rate (12.2% in the group that underwent an AI and the 25.8% in which an IVF was performed) and the live newborn rate between both groups (8.7 % in the group that underwent AI and 16% in which IVF was performed), both being higher for the group subjected to in vitro fertilization. In the twin pregnancy rate, we also found significant differences (p 0.005) between both groups, being 6.8% in the patients undergoing IVF compared to 0.8% in the inseminations.

Limitations, reasons for caution: Regarding the costs per cycle, indirect costs of these have not been taken into account, such as values in the cost of pregnancy and delivery of single or multiple pregnancies, or costs of complications derived from the techniques (ovarian hyperstimulation syndrome, fetal reductions, terminations for other reasons, etc.)

Wider implications of the findings: The recommendations made by the NICE promote IVF treatment to couples with infertility of unknown origin. In our opinion, this recommendation should be subjected to a more extensive cost-effectiveness analysis of both techniques, given that IVF requires a considerably higher investment of resources, finding results not so different as expected.

Trial registration number: Not applicable

P-727 Prevalence of ultrasound-detected gynaecological pathology in a One-Stop fertility clinic

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Study question: What is the prevalence of abnormal findings on transvaginal ultrasound scan (TVS) in a population of women presenting with subfertility to a One-Stop fertility clinic?

Summary answer: Two thirds of women in our population had ultrasound-detected pathology. The five commonest pathologies were uterine fibroids, polycystic ovaries, endometriosis, adenomyosis and benign ovarian cysts.

What is known already: Gynaecological pathology is common in women presenting with subfertility. However, their prevalence varies depending on the age, geography, background health of the population and study design. Few prevalence studies performed in the general female population show definitive associations with subfertility. As imaging techniques become increasingly sophisticated and patient demographics evolve over time, the prevalence of gynaecological pathology is anticipated to change. Understanding their prevalence in a subfertile population would shed light on the burden of disease, providing information about prevention strategies and service priorities. There are no published studies on the prevalence of ultrasound-detected gynaecological pathology in the subfertile population.

Study design, size, duration: This was a retrospective cross-sectional study of 1558 women presenting to a One-Stop fertility clinic of a university teaching hospital between January 2012 and December 2020.

Participants/materials, setting, methods: Women who attend the clinic routinely have their demographic data and a detailed clinical history taken prior to a transvaginal ultrasound scan. A clinical examiner trained in transvaginal ultrasonography performs the ultrasound examination in a standardized fashion. Ultrasound features and diagnoses are systematically recorded in an electronic database. We obtained demographic data and details of gynaecological diagnoses from the electronic database. We analysed the data using descriptive statistics and reported our results as proportions.

Main results and the role of chance: The median age of women at the ti me of scan was 35 years (range 21-46 years). The mean Body Mass Index was 24.8 kg/m2 (range 16.9-50.4 kg/ m2). The median duration of subfertility was 24 months (range 3-168 months). 472/1558 (30.3%, 95% CI 28.0-32.6) women had normal pelvic scans. The most frequent pathology seen in our population was uterine fibroids (410/1558, 26.3%; CI 24.1-28.6); 10.5% of these fibroids distorted the uterine cavity. Polycystic ovaries were the next most common pathology (363/1558, 23.3%; CI 21.2-25.4), followed by endometriosis (177/1558, 11.4%; CI 9.8-13.0), adenomyosis (160/1558, 10.3%; CI 8.8-11.9) and benign ovarian cysts (122/1558, 7.8%; CI 6.6-9.3). The other abnormalities seen on scan included congenital uterine anomalies (81/1558, 5.2%; CI 4.2-6.4), endometrial polyps (69/1558, 4.4%; CI 3.5-5.6), pelvic adhesions (44/1558, 2.8%; CI 2.1-3.8) and intrauterine adhesions (13/1558, 0.8%; CI 0.5-1.4).

Of the 1086 women with abnormal scans, 832 (76.6%, CI 74.0-79.1) had one pathology detected on TVS and 254 (23.4%, CI 20.9-26.0) had more than one pathology detected.

Limitations, reasons for caution: A limitation of our study was the lack of histological confirmation of the ultrasound findings. Due to our smaller sample size, our prevalence could potentially be overestimated.

Wider implications of the findings: The relevance of each pathology to chances of conception requires further examination to avoid under- or over-treating women in their fertility journey. Our findings may provide a background for future correlation studies. Furthermore, a scan quality assessment may be considered if the detection rate is substantially different in similar populations.

Trial registration number: Not applicable

P-728 Can in couples with unexplained infertility the use of a prediction model to triage assisted reproduction technology save costs?

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Study question: Can in couples with unexplained infertility a prognosis-tailored management strategy, that delays treatment if natural conception prospects are good, reduce costs without affecting live-birth rate?

Summary answer: In couples with unexplained infertility, use of a prognostic tool for natural conception followed by expectant management in good-prognosis couples is cost-effective.

What is known already: Few countries have guidelines for the assessment of the likelihood of natural conception to determine access to publicly funded ART. In the Netherlands and New-Zealand, couples with unexplained infertility who have a good prognosis for natural conception are encouraged to delay starting ART. However, the cost-effectiveness of this prognosis-tailored treatment strategy has not been determined.

Study design, size, duration: We studied couples with unexplained infertility to compare a prognosis-tailored strategy to care-as-usual. In the prognosis-tailored strategy, couples were assessed using Hunault's prediction model. In good-prognosis couples (12-months natural conception >40%), outcomes without ART were modelled by censoring observations after start of ART. We then assumed that poor-prognosis couples (<40% natural conception) were treated, while good-prognosis couples delayed the start of treatment for 12 months. Data for the care-as-usual model were based on real observations.

Participants/materials, setting, methods: We studied 272 couples with unexplained infertility. Costs of in vitro fertilisation (IVF) and intra-uterine insemination (IUI) were calculated based on the out-of-pocket costs and Australian Medicare costs. In a cost-effectiveness model, we compared costs and effects of both strategies.

Main results and the role of chance: The prognostic model classified 272 couples with unexplained infertility as favourable (N=107 (39.3%) or unfavourable prognosis (N=165 (60.7%)) for natural conception. In the prognosis-tailored strategy, the cumulative live-birth rate was 71.1% (95% CI 64.7% - 76.4%) while the number of ART cycles was 393 (353 IVF; 40 IUI). In care-as-usual strategy, the cumulative conception rate leading to live-birth for the cohort of 272 couples, who underwent a total of 398 IVF cycles and 48 IUI cycles, was 72.1% (95% CI 65.7% - 77.4%). Mean time to conception leading to live birth was 388 days in the prognosis-tailored strategy and 419 days in the care-as-usual strategy.

This translated for the 272 couples into potential savings of 45 IVF cycles and eight IUI cycles, which cost a total of AUD$ 125,817 for out-of-pocket and AUD$ 264,497 for Australian Medicare. The average cost savings per couple was AUD$ 1,435 (out-of-pocket AUD$ 463 per couple and Australian Medicare AUD$ 962 per couple). The incremental cost-effectiveness ratio, which was calculated as the total costs per additional live-births, was AUD$ 143,497 per additional live birth.

Limitations, reasons for caution: This study was limited to couples at a single IVF clinic. The modelling was also based on several key assumptions, particularly the number of fresh and frozen embryo transfer cycles for each couple.
Wider implications of the findings: Our results show that in couples with unexplained infertility the use of a prognostic model guiding the start of an IVF-treatment reduces costs without compromising live birth rates.
Trial registration number: Not applicable

P-729 Seasonality and lunar phase impact zona pellucida thickness while assisted reproductive treatment outcome shown no differences between seasons

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Study question: Does seasonal variation impact zona pellucida (ZP) thickness, other assisted reproductive treatment (ART) factors and ART outcome?
Summary answer: Seasonality and lunar phase impact ZP thickness while specific weather conditions alone do not, however, seasonality does not impact other ART factors or ART outcome. What is known already: Several epidemiological studies have demonstrated seasonal variation in natural pregnancy and birth rate, which varies across geographic regions. It has been suggested that temperature and light may affect the ability to conception via hormonal changes. However, data regarding the seasonal variation during ART is controversial and several studies with conflicting results have been published. One retrospective observational cohort study reported the significant influence of seasonality on fertilization rates with highest ones during the spring and the lowest ones in the autumn. However, another retrospective study did not demonstrate any significant influence of the seasons on ART outcome.
Study design, size, duration: This retrospective study was performed in the Fertility Center, VUH Santaros Clinics, Lithuania. 959 IVF/ICSI cycles conducted in IVF laboratory between 2017 and 2019 were analysed. The thickness of ZP was measured of 5002 oocytes retrieved between 2017 and 2018. Degenerated oocytes were excluded from the study. Average temperature (AT), precipitation (AP) and sunshine hours (ASH) of every month were taken from Lithuanian Hydrometeorological Service database. Lunar phase (LP) data was collected using Google Calendar.
Participants/materials, setting, methods: IVF/ICSI cycles were divided into four seasonal groups according to the day of oocyte pick-up. The number of retrieved and fertilized oocytes, transferred embryos, fertilization and pregnancy rates were compared among groups. Then, to avoid bias in fertilization rate, ICSI cycles were excluded and only IVF cycles were analysed. Measurements of ZP thickness were taken using NIS-Elements F software. It was evaluated if AT, AP, ASH, LP and seasonality had an effect on ZP thickness.
Main results and the role of chance: The mean number of retrieved oocytes and fertilized oocytes as well as the percentage of women who conceived was highest in the spring and lowest in the summer without statistical significance among all seasonal groups ($p>0.05$). The fertilization rate was lowest in the spring (66.60%) and highest in the autumn (68.76%) without statistical significance among all four groups. The odds were 1.49 times higher to conceive in spring compared to summer and this result was statistically significant (95% CI 1.01-2.21; $p=0.046$), however, when comparing all four seasons together, the difference was not significant. The calculations with only IVF cycles followed the same pattern except that the odds ratio results were not significant and the fertilization rate was highest in the winter. None of the weather conditions (average temperature, average precipitation and average sunshine hours) had an impact on ZP thickness. However, the mean ZP thickness was lowest in the summer (18.86 ± 3.08 μm) and highest in the autumn (19.43 ± 2.98 μm) and the difference among all four seasons was statistically significant ($p<0.05$). The mean ZP thickness was lowest during the first quarter lunar phase and highest during the new moon phase with statistical significance among groups ($p<0.05$).
Limitations, reasons for caution: A limitation of our study is unequal number of the IVF/ICSI procedures between months/seasons (e.g., the sample size of autumn was 340 while the sample size of summer was only 161). Also, the measurements of ZP were taken manually therefore there could be some errors.
Wider implications of the findings: Understanding possible effects of external factors on ART outcome is important for the best treatment results. Even though seasonality and lunar phase significantly impact ZP thickness, we could not demonstrate any significant seasonal influence on other ART factors or ART outcome. Further studies with higher number of patients are required.
Trial registration number: Not applicable

P-730 "Fertility Check Up": A proposal for assessment of women's fertility potential. Analysis and evaluation of the first 200 women

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Study question: Is there an interest in offering a fertility assessment to all women, with or without proven infertility, whatever their personal situation or parental project ?
Summary answer: Assessing the fertility of all women allows us to inform and advise them, in order to optimize their chances to achieve their parenting project.
What is known already: In a society where the age of childbearing is increasing and where women want to be able to postpone their pregnancies and to plan their parenting plan, there is no medical recommendation to assess fertility of women who are single or who do not have proven infertility.
Study design, size, duration: We implemented a new proposal in our reproductive medicine department, the "Fertility Check Up" (FCU), allowing any woman, whatever her personal situation or parental project, to benefit from an evaluation of her fertility, as well as personalized information and advice, to optimize the realization of her life plan.
Participants/materials, setting, methods: The FCU is carried out on female volunteers who do not need to be referred by a doctor. The fertility evaluation is performed by a self-questionnaire and an "all-in-one" ultrasound examination (Fertiliscan) including a complete pelvic ultrasound with a hystero-salpingo-foam-sonography (Hyfosy); this examination allows an anatomical and functional evaluation of the female reproductive system, in one step. Women then benefit from a personalized interview with a fertility specialist.
Main results and the role of chance: In the first year, 200 women aged 24 to 48 years old benefited from this examination, 56% of whom had never attempted to conceive. Anomalies found included: tubal diseases (29%), congenital or acquired uterine anomalies (11,5%), and endometriosis (6,5%). We concluded to a low ovarian reserve for age in 14% of cases. 84 % of women say they felt little or no discomfort during the Fertiliscan. A questionnaire was sent to women 6 months after the FCU: among the 85 women with a desire for pregnancy at the time of the FCU, 29.1% obtained a pregnancy, and 36% began ART procedures. Among the women who had no plans for pregnancy, 50% stated that the completion of the FCU had modified their personal or professional plans regarding a possible desire for future pregnancy.
Limitations, reasons for caution: Women are informed that the FCU gives them indications about their theoretical chances of pregnancy, but that there is no way to be sure that a woman will ever bear a child, as 10% of infertilities remain idiopathic.
Wider implications of the findings: The proposal of fertility assessment for women, whether infertile or not, with or without immediate pregnancy plans, allows for information, advice and treatment if necessary. Women are better informed about their own fertility, and can get the best chances to achieve their parental project, with, or ideally without, assisted-reproductive-techniques.
Trial registration number: Not applicable

P-731 Correlation between fertility rate, utilisation of ART and gross domestic product across Europe

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Study question: Are there any correlations between a country's wealth determined by GDP per capita and its total fertility rate (TFR) and utilisation of ART in Europe?
Summary answer: There is strong correlation across Europe between GDP and utilisation of ART. This correlation does not exist when only investigating the European Economic Area (EEA)
What is known already: The number of documented ART cycles has increased significantly from 203,893 cycles in 1997 (first European report) to 918,159 in 2016. During the same period, growth was observed in European GDP and, to a lesser extent, TFR following a significant and prolonged decline. Global data suggest that utilisation rate is higher in developed countries, speculated to be due to either generous reimbursement systems or higher affordability for patients paying out of pocket. This study analysed for the first time the relationships between national GDP, TFR and utilisation in Europe both as a whole, and specifically the more affluent EEA
Study design, size, duration: This study was an analysis of publicly available primary international reports: total cycles in the European IVF-monitoring Consortium (EIM) and TFR, GDP and population size from the World Bank indicators (https://data.worldbank.org/indicator). The period studied ranged from the first EIM report for 1997 (published in 1999) to the 20th report for 2016 (published in 2020).
Participants/materials, setting, methods: TFR was described as births per women (BPW) and country wealth was presented as GDP per capita in US Dollars. Utilisation rate was defined as the total national number of cycles (fresh IVF and ICSI, and frozen embryo transfer) divided per population, and presented as cycles per million (CPM). When utilisation was not reported, total cycles were projected by proportional calculation. Pearson Correlations were calculated using Sigmaplot for utilisation, GDP and TFR in 2016
Main results and the role of chance: Forty countries were included in the EIM report for 2016, of which 18 reported in full. The median utilisation rate was 1280 CPM (range 162 - 3,156) and median TFR was 1.6 BPW (range 1.26 - 2.73); only one country, Kazakhstan, had a TFR above the natural fertility replacement level of 2.1 BPW. Mean GDP was $31604 per capita (range $10,610 - $110,650). There was no correlation between TFR and utilisation or between TFR and GDP, however there was a significant positive correlation between GDP and CPM (correlation coefficient = 0.428; P = 0.00661). Compared to Europe as a whole, analysis of only the EEA countries – EU member states plus Norway, Iceland, and Switzerland – revealed a similar median TFR (1.59), but a 27% increase in the utilisation rate to 1629 CPM (range: 317 – 3157) and 24% rise in GDP per capita to $39,300 (range: $19,885- $110,650). For the EEA, no significant correlations were observed, including between GDP and utilisation (correlation coefficient = 0.131; P = 0.507). Additionally, there was no significant correlation between TFR and GDP in the EU for the period of 1997 – 2016.
Limitations, reasons for caution: The data is a snapshot of a single year, but we observed similar outcomes in previous years. Projection calculation of utilisation in partially reporting countries may cause bias, however, with a reporting level of 92% (1347 of 1467 clinics), this bias is probably very limited.
Wider implications of the findings: Findings of this study confirm that there are strong disparities in the availability of ART even in Europe. This difference does not exist in the more affluent countries in Europe suggesting that the reason for lower utilisation in lower-income countries being reduced affordability.
Trial registration number: NA

P-732 Maternal over-the-counter analgesics use during pregnancy and adverse perinatal outcomes: cohort study of 151,141 singleton pregnancies

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Study question: Is *in utero* exposure to five over-the-counter (non-prescription) analgesics (paracetamol, ibuprofen, aspirin, diclofenac, naproxen) associated with offspring health outcomes?
Summary answer: Consumption of over-the-counter analgesics during pregnancy, either as single compounds or in combinations, is significantly associated with a variety of adverse offspring health outcomes.
What is known already: A high percentage of pregnant women use over-the-counter analgesics during pregnancy globally. Some of these compounds such as paracetamol are considered safe to use, while contraindications exist for others, such as NSAIDs use beyond gestational week 30. Current evidence regarding the safety of use during pregnancy in humans is largely conflicting. Results from many published human studies on the topic suffer from limitations including use of small cohorts, short study time or failure to adjust for important confounders. These may explain conflicting results that cause significant concern regarding evidence-based prenatal guidance on use during pregnancy.
Study design, size, duration: Retrospective cohort study using the Aberdeen Maternity and Neonatal Databank. Data from 151,141 singleton pregnancies over 30 years (between 1985 and 2015) were used. Consumption of paracetamol, ibuprofen, aspirin, diclofenac and naproxen during pregnancy was recorded in medical notes of each woman. In our analysis, the control group was pregnancies where no analgesic was consumed, and the exposure groups included pregnancies with over-the-counter analgesic consumption either in combinations or as single compound use.
Participants/materials, setting, methods: Maternal baseline characteristics were compared using 2 tests for categorical variables and Mann-Whitney for continuous variables (significance at <0.05). Premature delivery, stillbirth, neonatal death, baby weight, neonatal unit admission, APGAR score at 1 and 5 minutes, neural tube defects, amniotic band defects, gastroschisis, and, in males only, hypospadias and cryptorchidism, were the outcomes assessed. Crude (cORs) and adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were calculated using logistic regression to control for confounders.
Main results and the role of chance: The overall prevalence of over-the-counter analgesics use during pregnancy was 29.1%, increasing over the 30-year study period, to over 60% of women in the last seven years of the study. 83.7% of those women reported first trimester use when specifically asked at their first antenatal clinic visit. Pregnancies exposed to at least one of the five analgesics were independently associated with increased risks for premature delivery <37 weeks (aOR=1.50, 95%CI 1.43-1.58), stillbirth (aOR=1.33, 95%CI 1.15-1.54), neonatal death (aOR=1.56, 95%CI 1.27-1.93), birthweight <2,500g (aOR=1.28, 95%CI 1.20-1.37), birthweight >4,000g (aOR=1.09, 95%CI 1.05-1.13), admission to neonatal unit (aOR=1.57, 95%CI 1.51-1.64), APGAR score <7 at 1 minute (aOR=1.18, 95%CI 1.13-1.23) and 5 minutes (aOR=1.48, 95%CI 1.35-1.62), neural tube defects (aOR=1.64, 95%CI 1.08-2.47) and hypospadias (aOR=1.27, 95%CI 1.05-1.54 males only).). Associations of paracetamol alone with high birth weight, neural tube defects and hypospadias were not significant in the adjusted analysis. Diclofenac consumption was associated with significantly decreased odds of stillbirth (aOR=0.59, 95%CI 0.41-0.87).
Limitations, reasons for caution: Our data were based on medical notes; however, consumption is self-reported, and details on the timing, dosage, product type (single-ingredient vs combination) and administration type were not available in the database. Our study only considered neonatal health outcomes and longer-term follow-up of the offspring was not available at this time.
Wider implications of the findings: This is one of the largest and most comprehensive studies into analgesic use in pregnancy. The increased risks of adverse neonatal outcomes associated with non-prescribed, over-the-counter, analgesics use during pregnancy indicate that healthcare guidance for pregnant women regarding analgesic use should be re-assessed.
Trial registration number: N/A

P-733 In infertile patients, risk factors for environmental reprotoxic exposure are widespread, limited in number and modifiable

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Study question: What are the risk factors for environmental reprotoxic exposure in infertile patients?
Summary answer: The most represented categories of reprotoxic risk factors (RRF) were dietary exposures (86% of patients), overweight (46%), psychoactive substances (38%) and male occupational exposures (63%).
What is known already: Numerous studies have reported the deleterious effects of environmental reprotoxic exposures on male or female fertility.

These studies most often focus on the impact of a limited number of reprotoxic risk factors (body mass index (BMI), dietary habits, tobacco or alcohol consumption) or a limited number of chemical or physical reprotoxic exposures: phthalates, occupational exposures, or pesticides.

Despite the call of several reproductive health professional societies and public health agencies for taking environmental health into account in women of childbearing age, this approach remains little realized in current practice.
Study design, size, duration: We conducted a prospective, monocentric study between June 2018 and February 2020 in women and men visiting the fertility unit of our University Hospital for assisted reproduction technique (ART) treatment.
Participants/materials, setting, methods: Patients completed a self-questionnaire to collect information about i) the various types of exposure to RRF, and ii) frequency and intensity of exposures (qualitative and semi-quantitative approach).

We performed a literature search in order to define the environmental factors and the exposure level thresholds associated with a "recognized" or "suspected" RRF and we analyzed their nature and number in patients.
Main results and the role of chance: During the inclusion period, we received 545 couples in consultation, and 405 were included in this study (810 patients/1090, participation rate: 74%). 65% of women and 68% of men self-reported at least one "recognized" RRF. In men, they were from exposure to solvents, heat, psychoactive substances and a BMI>25; in women, from exposure to poor indoor air quality, psychoactive substances and a BMI>25. A limited number of recognized risk factors were recorded in the majority of patients (one, two or three risk factors in 65% of patients).

Men were more often exposed than women to occupational risk factors (63% of men versus 28% of women) such as solvents and ambient heat, and women were more often exposed than men to poor indoor air quality and volatile organic compounds (49% of women versus 30% of men). We note that the majority of the risk factors for reprotoxic exposure found were modifiable, specifically dietary, occupational, overweight and psychoactive substance exposures.
Limitations, reasons for caution: One limitation is the collection of data via a self-administered questionnaire, which makes it possible to estimate the risk factors for reprotoxic exposure, but not to detect them in a measurable way, for example, through exposure biomarkers. In addition, no data is available about all RRF in the general population.
Wider implications of the findings: We suggest that if the individual screening of each infertile patient's RRF was done before ART, most patients could act on a limited number of modifiable RRF, in the aim of increasing their chances of natural pregnancy and improving ART outcomes.
Trial registration number: N° 2018-13-06-004 (Ethics Committee of the University of Aix Marseille) and N° 2020-27 (Assistance Publique - Hôpitaux de Marseille General Regulation on Data Protection).

P-734 Parenthood among men diagnosed with cancer in childhood and early adulthood – trends over time in a Danish national cohort

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Study question: Is the rate of fatherhood among men diagnosed with cancer in childhood and early adulthood different from men without cancer – have differences changed over time?
Summary answer: Men diagnosed with cancer had significantly reduced rates of fatherhood compared with undiagnosed men. Rates of fatherhood among the cancer survivors increased markedly over time.
What is known already: The number of children and young adolescents who survive cancer has steadily increased over the past decades, with a current 5-year survival rate of approximately 80%. Consequently, life circumstances after cancer have gained increasing importance, including the desire among survivors to have children and a family. MAR technologies to aid reproduction among cancer survivors have been developed, and fertility preservation is increasingly a topic being discussed before undergoing cancer treatment. But the potential for fertility preservation differs depending on age at diagnosis and type of cancer. Earlier studies have shown decreased fertility rates among survivors of childhood and adolescent cancer.
Study design, size, duration: This study is a national, register-based cohort study. Men diagnosed with cancer in childhood and early adulthood (<30 years of age) were registered in the Danish Cancer Register in 1978-2016 (n= 15,600). At time of diagnosis, each cancer-diagnosed man was randomly age-matched with 150 undiagnosed men from the background population within the same birth year. The men were followed in medical registers and socio-demographic population registers until death, migration or end of study December 31st, 2017.
Participants/materials, setting, methods: Fatherhood among the boys and young men diagnosed with cancer was compared with the age-matched comparison group in all statistical analyses. Cancer diagnoses were categorized as central nervous system (CNS), haematological cancers or solid cancers. Also, analyses were stratified by age at diagnosis (0-9, 10-19, 20-29 years) and year of diagnosis (1978-89, 1990-99, 2000-16). Death was incorporated as a competing risk in all analyses.
Main results and the role of chance: The study population consisted of 15,600 boys and young men diagnosed with cancer between 1978 and 2016 and 1,386,493 men in the age-matched comparison group. Men surviving CNS cancer had the lowest hazard ratio of fatherhood compared with the age-matched comparison group (HR= 0.64, 95% CI 0.57-0.73), followed by survivors of haematological cancers (HR= 0.90, 95% CI 0.82-0.98) while the highest chance of fatherhood was slightly increased among survivors of solid cancers (HR= 1.13, 95% CI 1.10-1.16). The hazard ratio of becoming a father increased over time. From the first decade to the last decade 30 years later, the hazard ratio of becoming a father increased for solid tumours (HR 0.76, 95% CI 0.72-0.80 to HR 1.07, 95% CI 0.96-1.19), haematological tumours (HR 0.60, 95% CI 0.51-0.71 to HR 0.97, 95% CI 0.76-1.23) and CNS tumours (HR 0.47, 95% CI 0.39-0.58 to HR 1.04, 95% CI 0.56-1.93) compared to the age-matched comparison group. Also, men diagnosed with cancer when aged 20-29 years more likely became fathers over time (HR 0.79, 95% CI 0.74-0.84 to HR 1.09, 95% CI 0.98-1.22).
Limitations, reasons for caution: The study was based on register data, and information was not available about the men's fertility potential, whether they had a desire to have children and whether it was possible for them to find a partner. Also, information about fertility preservation, e. g. sperm freezing, could have provided additional insights.
Wider implications of the findings: Information and education of male patients diagnosed with cancer about fertility preservation options, and chances to create their own family is crucial. Reassuringly, time trends showed more men with a previous cancer diagnosis becoming fathers in recent years than earlier, reflecting that survival and fertility preservation have improved over time.
Trial registration number: N/A

P-735 Spontaneous pregnancies among infertile couples during assisted reproduction lockdown for COVID-19 pandemic

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Study question: Evaluate the population attending Assisted reproductive techniques (ART) who have suffered the interruption of fertilization paths because of the SARS-CoV-2 italian national lockdown declared under goverment provision.

Summary answer: The research show that some infertile couples candidate to ART paths obtained a spontaneous pregnancy during the lockdown due to SARS-CoV-2 pandemic.

What is known already: The real impact of psychological stress on couple infertility in terms of pregnancies obtained is largely under-investigated in the literature and the potential low frequency of sexual activity is usually poorly considered in the management of couple infertility and its role on pregnancies failure is unclear, but probably underestimated. Moreover, the worldwide spread of the SARS-CoV-2 infection has profoundly affected all aspects of human life, with tangible consequences in several contexts, including reproduction. This allowed to highlight the interrelation between psychological distress and reproductive medicine are still conflicting.

Study design, size, duration: This is a study conducted at the Fertility Centre of the Department of Obstetrics and Gynaecology of Reggio Emilia (Italy), that evaluated the conception ability of couples who suffered the postponement of ART cycles during the SARS-CoV-2 pandemic. In particular, we collected anamnestic, anthropometrical and demographic data of those women attending ART straddling the lockdown period, from March 2020 to April 2020.

Participants/materials, setting, methods: The study evaluated couples attending ART, who had an interruption of the fertilization paths due to the SARS-CoV-2 pandemic. The variables as women age, BMI, duration of infertility, number of sexual intercourses per week and infertility aetiology were considered in a multivariate logistic analysis. The statistical analysis was performed setting pregnancy (categorical data) as the dependent variable, and all other available data as either covariates or cofactors.

Main results and the role of chance: Among the 431 couples recalled to reschedule ART cycles 34 couples (7.9%) obtained a spontaneous pregnancy during the COVID-19 lockdown. The statistical analysis for the 431 couples recalled showed that average duration of couple infertility was 3.5 ± 2.8 years, while an exclusively female causal factor was observed in the 23.7% of cases (102 couples), an exclusively male one in the 32.7% (141 couples), a coexistence of male/female factor in the 18.6% (80 couples), and an idiopathic form in the 25.1% (108 couples). The 34 couples obtained a spontaneous pregnancy presented a female infertility factor in the 26.5% of cases (9 couples), a male factor in the 29.4% (10 couples), a male/female factor in the 11.8% (4 couples), and idiopathic infertility in the 32.4% (11 couples). The male factor of infertility was not evaluated in all couples, thus this definition came from the anamnestic evaluation of the couple. In a multivariate logistic analysis we highlighted that the infertility history duration and the sexual intercourses frequency were significantly related to pregnancy (F=4.8, degrees of freedom=1, p=0.030 and F=81.6, degrees of freedom=1, p<0.001, respectively).

Limitations, reasons for caution: Despite the short observation period (two months of italian national lockdown declared under goverment provision), the large sample size of women evaluated attending to a single ART centre constitutes a point of strength of our study. However, the absence of a control group represents the most important limit.

Wider implications of the findings: In conclusion, the lockdown allowed to increase the knowledge about under-explored causes of idiopathic infertility: the frequency of sexual intercourses. This aspect in reproductive medicine could help to identify those couples able to conceive spontaneously, avoiding unnecessary over-treatment, and to correctly apply ART to those couples who really need it.

Trial registration number: none

P-736 Elevated levels of ambient air pollutants increase the primary sex ratio in human embryos

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Study question: Are there any associations between ambient outdoor air pollution and the primary sex ratio (PSR)?

Summary answer: Short-term exposure to increased PM10, PM2.5 and NO2 levels were significantly associated with higher PSR.

What is known already: PSR estimates represent a backward extrapolation from data based on spontaneous or induced abortions, fetal deaths or live births and are usually male-biased. A recent study, analyzing 3- to 6-day-old embryos derived from assisted reproductive technology (ART) procedures, showed that the sex ratio at conception is unbiased (0.5). Epidemiologic studies of air pollution on secondary (birth) sex ratio showed that higher levels of particulate pollution were associated with increased rates of female birth. However, a direct association between urban levels of air pollutants and PSR has not been reported.

Study design, size, duration: A retrospective cohort study was carried out to assess the impact of long- or short-term exposure to six ambient outdoor air pollutants (particulate matter, PM10µm and PM2.5µm; SO2; CO; NO2; O3) on PSR (XY/XX) of couples undergoing their first IVF cycle for preimplantation genetic screening (N=337). Data was from fixed air quality monitoring stations across the city between January 2014 and December 2018. Embryos with sex chromosome abnormalities were excluded from the analysis.

Participants/materials, setting, methods: Average concentrations of the pollutants for the 90 (long-term exposure) and 15 days (short-term exposure) predating oocyte retrieval represented the exposures of interest. Pollutant levels were categorized into quartiles (Q1 to Q4) and exposure risk was divided into two periods in which average concentrations and confidence intervals for the pollutants were in the upper quartile (Q4 period) or not (Q1-Q3 period). The strength association between exposure risk and PSR was performed through analysis of covariance.

Main results and the role of chance: The estimated means of PM10, PM2.5, SO2, NO2, O3 and CO for Q1-Q3/Q4 periods were 27.7/39.3, 16.7/23.7, 2.5/3.9, 37.0/46.4, 32.2/45.3 µg/m3 and 0.64/0.87 ppm and 26.3/43.0, 16.0/26.3, 2.4/4.2, 36.5/47.8, 31.7/50.4 µg/m3 and 0.62/0.90 ppm for long- and short-term exposures, respectively. PM10, PM2.5 and NO2 levels in the Q4 period had significantly higher PSR (138.1, 134.0 and 137.6) when compared to Q1-Q3 period (94.4, 98.1 and 96.4) for the short-term exposure (*p=0.0193*; *p=0.0439*; *p=0.0180*, respectively). PM10, PM2.5, SO2, NO2 and CO levels in the Q4 and Q1-Q3 periods for the long-term exposure showed no significant effect on PSR. Contrastingly, O3 levels in the Q4 period had significantly lower PSR (82.6) when compared to Q1-Q3 (115.9) for the long-term exposure (*p=0.0202*). A monotonic increase in PSR was observed with increased PM10 concentration in the Q4 period for the short-term exposure (F-ratio: 4.4476; *p=0.0352*).

Limitations, reasons for caution: Some limitations of the study should be underlined, such as its retrospective nature, exposure assessment based on pollutant levels derived from a network average across city sites, and limited extrapolation of the results to the general population.

Wider implications of the findings: Our data suggest that short-term exposure to environmental factors could affect the primary sex ratio in polluted

seasons or cities. A monotonic effect on PSR in the case of exposure to increasing PM10 levels was identified.
Trial registration number: Not applicable

P-737 Effect of dietary patterns on clinical pregnancy and live birth outcomes in men and women receiving assisted reproductive technologies: a systematic review and meta-analysis

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Study question: Is there a relationship between dietary patterns and clinical pregnancy or live birth outcomes in men and women of reproductive age undergoing assisted reproductive technologies (ART)?
Summary answer: While the Mediterranean and pro-fertility diets show potential to improve fertility outcomes, the association between dietary patterns and ART success is currently inconsistent.
What is known already: The nutritional status of reproductive-aged couples can have a significant impact on fertility. While the consumption of individual foods and nutrients are known to influence reproductive success, the effect of dietary patterns on clinical pregnancy and live birth outcomes in people using assisted reproductive technologies (ART) is currently unknown.
Study design, size, duration: Six electronic databases were systematically searched for original research published in English between January 1978 and December 2020 reporting on the effect of pre-defined dietary patterns on either clinical pregnancy and/or live birth rates following invitro fertilisation or intracytoplasmic sperm injection in men and women aged 18-49 years. Screening of all retrieved articles was performed independently by two review authors. Eligible studies underwent quality assessment and qualitative and quantitative synthesis using random-effects model meta-analyses.
Participants/materials, setting, methods: Studies eligible for inclusion in this systematic review were cross-sectional, cohort, clinical trial, and randomised controlled trial study designs. Eligible participants were both males and females, aged 18-49 years, who were undergoing invitro fertilisation or intracytoplasmic sperm injection. Studies were excluded if their primary analysis assessed consumption of individual foods, food groups, vitamins, or minerals, rather than dietary patterns. Studies measuring proxy estimates of fertility status such as sperm quality or quantity were not included.
Main results and the role of chance: Twelve studies (11 prospective cohort studies, 1 randomised controlled trial) reporting on 3144 participants (92% female) were included in the review. Six studies were of positive methodological quality, and six were of neutral quality. Eleven studies used validated food frequency questionnaires to quantify dietary pattern adherence. In individual studies, three dietary patterns (Mediterranean diet, pro-fertility diet, Iranian traditional medicine diet) were associated with increased likelihood of achieving a clinical pregnancy, while two dietary patterns (pro-fertility diet, Mediterranean diet) were associated with increased probability of live birth. Meta-analyses of five Mediterranean diet cohort studies showed no association between dietary pattern and clinical pregnancy (OR 1.3; 95% CI: 0.73- 1.72, P=0.59), and meta-analysis of three Mediterranean diet cohort studies found no relationship between dietary pattern and live birth (OR 1.51; 95% CI: 0.83-2.76, P=0.18).
Limitations, reasons for caution: Males were under-represented in the included studies, and half of the studies were of neutral methodological quality. All studies completed dietary assessments at baseline only, however dietary assessments should ideally be undertaken at regular intervals throughout the duration of cohort studies, in the event that dietary patterns change over time.
Wider implications of the findings: Further research utilising higher quality nutrition research methodologies is required to better understand the association between dietary patterns and fertility outcomes during assisted reproductive technologies.
Trial registration number: Not applicable

P-738 Fertility Preservation: Comparative analysis about the knowledge of the topic between two female populations in Argentina and the United States

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Study question: Is there any difference in the knowledge that women in Córdoba (Argentina) and South Carolina (United States) have about fertility preservation, according to their socioeconomic and educational level?

Summary answer: Scarce knowledge about fertility preservation in both populations was registered. Only sectors of higher socioeconomic and educational level responded correctly with values close to 60%.
What is known already: There are numerous factors that may motivate the need to preserve fertility in young individuals. These factors can be grouped into two causes: social (postponement of motherhood, gender change, etc.), or medical (oncological or surgical treatments). In these situations, it is important that society in general has access to information about fertility and the possibilities of preserving it, if necessary. On the other hand, in each country and region in particular the information on this topic is distributed in different ways, which could generate differences in the level of knowledge on these issues in different population groups.
Study design, size, duration: Descriptive quantitative study. A total of 3,041 answers were obtained, 88.8% from Argentina and 10.00% from the United States. An 83.72% (2,521) of the answers were made by women.
Participants/materials, setting, methods: A closed-ended questionnaire of 20 questions was designed (segmentation and aspects of knowledge about fertility and its preservation) and distributed to different sectors of society through social networks. The survey was answered by people from Córdoba (Argentina) and South Carolina (US), of both sexes and different age groups, educational levels (basic/higher) and socioeconomic levels (medium-low/high). All answers were collected through SurveyMonkey and were analyzed using calculation programs and statistical tools (Excel 2016, Statistica 8.0).

Main results and the role of chance: Data showed that the 47.98% of Argentine women and 42.68% of American women surveyed do not know the age at which fertility begins to decline. The group with the highest percentage of incorrect answers (61.11%) for this question was that of Argentine women who had no previous experience with assisted fertility and come from the lower-middle social class. When asked about the knowledge about the factors that affect fertility, only 55% of Argentine women answered correctly, compared to 64.85% of American women. For the Argentine group, the proportion of correct answers increased to 62.23% for higher education level and to 56.60% for higher socioeconomic level. Regarding whether they know what fertility preservation procedure consists of, only 47.98% of Argentine women and 42.68% of American women answered correctly. On the other hand, 69.37% of the former and 63.18% of the latter do not know which biological materials can be cryopreserved. Finally, only 25.68% of women in Argentina know about the extent of their medical coverage in terms of fertility preservation procedures, while this percentage is 7.95% for the US population.
Limitations, reasons for caution: The comparison between the two countries may be challenged by the inequality in the response rate to the survey. However, even the smaller number of responses obtained in the USA is sufficient to obtain valid conclusions.
Wider implications of the findings: The level of misinformation registered in this study could imply reduced chances of achieving pregnancy in the future, especially for older women, those who wish to postpone motherhood or those who must undergo cancer treatments. This work provides important information in the politics designing promoting information access on fertility preservation.
Trial registration number: .

P-739 Fertility and its Preservation: Comparative Analysis about the Knowledge between Two Populations of Doctors and Health Professionals from Argentina and the United States

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Study question: Is there any difference in the knowledge that doctors and health professionals from Córdoba (Argentina) and South Carolina (USA) have about fertility preservation or about when it should be applied?
Summary answer: Both populations have enough knowledge about some aspects of fertility preservation, but its training must be improved so they can give adequate counseling.
What is known already: During the last decades, it has been observed that more young individuals need/decide to preserve fertility, whether for social or medical reasons. This presents a new challenge for the medical community, since, faced with this situation, it is important that society in general has access to information about fertility and the possibilities of preserving it, if necessary. To this end, it is essential that doctors and other health professionals have valid knowledge of the subject and are able to communicate it to their patients.
Study design, size, duration: Descriptive quantitative study. A total of 721 answers were obtained, 88.7% from Argentina and 11.3% from the United States. 28.43% (205) were doctors and 71.57% (516) were other health professionals.
Participants/materials, setting, methods: A closed-ended questionnaire of 20 questions was designed (segmentation and aspects about fertility preservation) and distributed to society through social networks. The survey was answered by people from Córdoba (Argentina) and South Carolina (US), of both sexes and different age, educational and socioeconomic levels. Only those with a medical degree or involved in some medical-related activity were selected. All answers were collected through SurveyMonkey and analyzed using calculation programs and statistical tools (Excel-2016, Statistica 8.0).
Main results and the role of chance: Data showed percentages of correct answers greater than 70% in all groups for the questions that analyze what factors can affect fertility, what situations can determine the need to preserve it, and what is the appropriate age for a woman to cryopreserve her eggs. On average, 82.4% of doctors and 72.87% of other health professionals know when it is the right time for patients diagnosed with cancer to receive information about the possibility of preserving their fertility. However, on average between both countries, only 34.63% of doctors has information about the legal medical coverage of their patients, while the 39.51% is completely unaware of their country's laws. Finally, the percentages of professionals who do not know what material can be cryopreserved in girls who need to undergo oncological treatments reach 46.34 and 64.33% (doctors and other health professionals respectively).
Limitations, reasons for caution: The comparison between the two countries may be challenged by the inequality in the response rate to the survey. However, even the smaller number of responses obtained in the USA is sufficient to obtain valid conclusions.
Wider implications of the findings: Both populations have sufficient information about factors which affect fertility and its preservation, especially in cancer situations. Misinformation in health personnel about these aspects directly affects possibilities of achieving future pregnancies for patients. Continuous updating and guidance should be a priority, as well as information dissemination and adequate medical counseling.
Trial registration number: .

P-740 Socio-cultural and clinical implications of 'routine' AMH testing in India: Insights from an interview study with the healthcare professionals (HCPs)

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Study question: How do Indian healthcare professionals describe their clinical experience with and perspectives on AMH testing in Indian women seeking fertility treatments including fertility preservation?
Summary answer: The HCPs cautioned against AMH testing as a screening tool in presumed fertile Indian women due to its anticipated impact on women's arranged-marriage prospects.
What is known already: AMH test is being increasingly used to assess women's ovarian reserve (OR) while planning fertility treatments or to guide decisions about fertility preservation (FP). There is weak evidence suggesting that serum AMH level and fertility treatment outcomes vary in different population groups. Surveys with women in reproductive age (e.g. the US, Ireland, the Netherlands) indicate that a majority wants to know their OR to aid reproductive decision making. As yet, both globally and in an Indian context, there are only few qualitative studies exploring the views of HCPs on the OR assessment in clinical practice and its socio-cultural implications.
Study design, size, duration: This paper reports the findings of an exploratory qualitative research aimed at understanding whether and how elective fertility preservation could influence reproductive autonomy of Indian women. Between June 2018 and April 2019, IVF specialists and obstetricians practicing in ten cities across five Indian states were interviewed in English (language commonly spoken) using a semi-structured interview guide. The discussion about OR assessment with AMH-testing was initiated by the participants indicating its significance in their clinical practice.
Participants/materials, setting, methods: The study sample included 17 male and 15 female HCPs, the majority (18/32) was practicing in Mumbai. Twenty-six of them were in private practice while six worked as OBGYNs in publicly funded teaching hospitals. Twenty-six participants were interviewed in their clinics and the remaining six using Skype or telephone. After several rounds of immersive reading, the interview sections on OR and AMH-test were analyzed inductively using Braun and Clarke's thematic analysis.
Main results and the role of chance: Several participants reported that many of their patients present with decreased OR (DOR) at a younger age and need higher dosages of hormones for ovulation induction compared to the dosages mentioned in international guidelines. They corroborated this experience with a few peer-reviewed articles indicating a six-years age difference in OR of Indian women undergoing IVF compared to Spanish women. A majority of participants advocated for the rational use of OR assessment in IVF patients but warned against its indiscriminate use or interpretation out of context due to concerns about overdiagnosis of ovarian factor infertility and overtreatment with IVF with donor eggs. Although the physicians who had performed elective FP perceived AMH test as a simple, affordable and empowering tool to guide FP decisions, most participants were critical of using AMH-test as a screening tool in young, presumed fertile women completing university education. They were concerned that a diagnosis of DOR as a result of such screening in this population in the Indian context will adversely impact women's chances of marriage and might further increase pressure on women to get married and complete their childbearing early even if they are not ready for it.
Limitations, reasons for caution: This is the first qualitative study assessing views of Indian HCPs on AMH testing. These results are indicative rather than a representation of views of Indian HCPs. Almost half of the contacted HCPs did not respond to interview requests; we do not know whether they had different views.
Wider implications of the findings: The insights on clinical implications of AMH testing in India are relevant to other societies beyond the Euro-American and Australian context where AMH testing will increase in the future. The socio-cultural implications of 'routine' AMH testing in India urges us to be aware of similar implications in other cultural contexts.
Trial registration number: Not applicable

P-741 Fetal exposure to maternal perceived stress and male reproductive function in a cohort of young adults

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Study question: Is exposure to maternal perceived stress during pregnancy associated with reproductive function in adult male offspring?
Summary answer: While maternal perceived stress was prevalent in the first trimesters of pregnancy, our preliminary findings indicate little association with reproductive function in young men.
What is known already: Though studies in animals point to a connection between prenatal exposure to maternal stress and reproductive function in offspring, the underlying biological mechanisms generating a deficit remain largely unclear. In humans, the few available studies focus on exposure to bereavement or other relatively strong objective stressful life events. Our individual perception of stress is, however, more likely the sum of a complex process involving both the actual input, previous experiences, coping strategies and support from our surroundings.
Study design, size, duration: Young men and their mothers were identified through records from the Danish National Birth Cohort (DNBC). Information on exposure, i.e. maternal perceived life and emotional stress, was available from telephone interviews conducted at approximately 30 weeks of gestation (1996 to 2001). Recruitment of the young men lasted from 2017 to 2019 with 1058 participants enrolled in the final FEPOS cohort.
Participants/materials, setting, methods: Each of the 1058 men in the FEPOS cohort completed an online questionnaire and clinical examinations and provided a blood and semen sample. Information on potential pre- and postnatal confounders was retrieved from the DNBC, the Danish National Patient Register and the Danish Medical Birth Register. We applied negative binomial regression models to examine associations between maternal perceived life and emotional stress scores and semen quality, testicular size and reproductive hormones among the young men.
Main results and the role of chance: Among the 1052 young men included in preliminary analyses, the majority was exposed to maternal perceived life and/or emotional stress (76% and 83%, respectively) during the first trimesters. Life stress was predominantly related to the actual pregnancy (48%), maternal disease (19%) or occupational conditions (33%). Emotional stress included especially being touchy (58%), sad (38%) or tense (36%), covering aspects of both stress, depression and anxiety. Overall, results indicate little association between maternal stress scores and measures of semen quality and testicular size. Our study involves a large cohort with prospectively collected exposure data and direct measures of several male reproductive outcomes. We applied inverse probability weighting to account for selection into the FEPOS cohort and included a range of *a priori* selected maternal confounders in our models.
Limitations, reasons for caution: The male fetus may be particularly sensitive to exposure during the differentiation of reproductive tissues (8-14 weeks of gestation). Our self-reported measures of exposure cover the first 30 weeks of gestation. Absence of association may, thus, be due to a lack of specific information on timing of symptoms.
Wider implications of the findings: While our preliminary findings may appear reassuring, further efforts to improve our understanding of maternal stress in relation to fetal health and potential consequences later in life are needed.
Trial registration number: not applicable

P-742 Medical contraceptive use in the French population: Can we explore it based on the national health insurance data?

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Study question: Are French national health insurance data reliable for studying the use of medical contraception?
Summary answer: Health insurance data produce a measurement of contraceptive use consistent with population-based survey data, which affords new opportunities for studying contraception.
What is known already: Medical contraception is a major public health issue as most women of reproductive age use it. It is usually studied through population-based surveys. However, such surveys are conducted only every 10 years, and analyses are limited by their sample size. French national health insurance data provide comprehensive and time-continuous information on each reimbursed contraceptive. However, because these data have been collected for a different purpose (reimbursement), their relevance for measuring the use of contraceptives needs to be assessed.
Study design, size, duration: Two sources were analysed. First, a cross-sectional cohort was extracted from the health insurance database, which includes all health reimbursements (such as those for medical contraceptives) and covers 98% of the French population, including 14 million women aged 15–49. Secondly, we used the last French survey on contraception, a cross-sectional study including 4,508 women aged 15–49 interviewed by phone.
Participants/materials, setting, methods: From both sources, we selected all women aged 15–49 living in metropolitan France. We identified the last medical contraceptive purchased by each woman between 2014 and 2019. The woman was then classified as currently using this contraceptive if the recommended duration of use for this contraceptive was still ongoing on 31 December 2019. Prevalences were compared to those observed in the population based survey.
Main results and the role of chance: Among the 14.3 million women aged 15–49 living in metropolitan France covered by the health insurance, 26.0% were using the pill, 17.4% an IUD (7.6% hormonal IUD; 9.9% copper IUD), and 3.1% an etonogestrel implant. These proportions are very close to and not statistically different from those observed in the population-based survey (26.2% for the pill, 18.4% IUD, and 3.1% implant). Contraceptive use varied widely with women's age. At ages 20–24, the most widely used contraceptive was the pill (42.2%), and very few long-acting contraceptives were used (7.6% IUD; 4.9% implant). At ages 30–34, the pill was less frequently used (21.6%) and IUD more frequently used (copper IUD: 15.79 %; hormonal IUD: 7.06%).
Limitations, reasons for caution: It cannot be ruled out that some contraceptives were purchased but never used and that a few women stopped using the contraceptive before the end of its recommended duration.
Wider implications of the findings: To our knowledge, this study is the first to estimate prevalence for Copper IUD and for hormonal IUD in France. Using the national health insurance database, it is now possible to monitor the use of each type of medical contraceptive over time in a reliable population-based approach.
Trial registration number: not applicable

P-743 The fertility paradox: the need for contraception after in vitro fertilisation

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Study question: What are a woman's contraceptive needs after successful in vitro fertilisation (IVF) pregnancy? and how should services respond to help prevent unintended pregnancies?
Summary answer: Women who have IVF pregnancies require tailored, postnatal contraception counselling. Services must provide evidence-based information about the risks of spontaneous conception to engage them effectively.
What is known already: Women undergoing IVF are an increasingly heterogeneous group with a wide range of causative subfertility factors. Furthermore, increasingly, women are accessing treatment primarily for reasons *other* than subfertility. The evidence relating to rates of spontaneous conception post assisted conception varies widely according to population, cause of subfertility, type and outcome of fertility treatment and length of follow-up. A recent large retrospective UK cohort study estimated the treatment-independent live birth rate after IVF live birth over 5 year follow up as 15% [https://doi.org/10.1093/humrep/dez099]. We aim to explore the experiences and views about contraception among this diverse group of women.
Study design, size, duration: A qualitative study of the views of women who have had spontaneous pregnancies after successful IVF was conducted in

September/October 2020. A qualitative approach of in-depth interviews was chosen to allow exploration of individual experiences in an area not much studied previously. The sample consisted of twenty interviewees from around the UK.
Participants/materials, setting, methods: Purposive and snowballing sampling methods were used with eligible participants recruited from a range of sources including social media and peer networks. The sample included a wide range of spontaneous pregnancy outcomes after successful IVF, including single and multiple livebirths, miscarriage, ectopic pregnancy and termination of pregnancy. The framework method was used for analysis using NVivo12 software.
Main results and the role of chance: Contraceptive choices were subject to a complex and dynamic interaction of influencing factors including i) beliefs regarding their own subfertility, ii) desire for more children and iii) their views on contraception. After IVF pregnancy, the majority of women (n=15) used no contraception or ineffective methods (inconsistent condom use or withdrawal) before their next pregnancy with only two women using hormonal methods (progesterone-only pill). Spontaneous pregnancy was not universally welcomed in this group and the inter-pregnancy intervals were often short (n=15, less than 18 months) or very short (n=6, less than 12 months). After subsequent spontaneous pregnancy, use of contraception and the most effective (long-acting reversible) methods remained low. Women held persistent beliefs regarding their subfertility despite subsequent spontaneous pregnancy (or pregnancies). Women associated aspects of the IVF process (e.g. need for multiple cycles, low numbers of eggs collected etc.) with a sense of personal failure, despite an ultimately "successful" outcome resulting in livebirth. This may contribute to or reinforce their self-belief in subfertility. Other specific barriers to contraception use, in women having IVF, included lack of knowledge of the likelihood of spontaneous pregnancy, lack of contraceptive experience and inherent incentives towards shorter inter-pregnancy intervals.
Limitations, reasons for caution: There is potential recall bias with some women recalling experiences associated with IVF treatment more than ten years ago. However our sample included women who were currently pregnant as well as women who were further towards the end of their reproductive life to capture a range of experiences.
Wider implications of the findings: The contraceptive needs of women having IVF pregnancies are being overlooked. Fertility services should take responsibility for providing accurate information on the risks of subsequent spontaneous pregnancy in this population. Maternity and community healthcare professionals must address women's perceptions of their fertility in order to engage them in contraception counselling.
Trial registration number: N/A

POSTER VIEWING
REPRODUCTIVE SURGERY

P-744 The features expression of some lymphocyte markers in the pelvic peritoneal adhesions' tissue at reproductive age women

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Study question: To study the expression of CD4, CD8, CD20, CD 138 in the tissue of the pelvic peritoneal adhesions at women of reproductive age.
Summary answer: Immunohistochemical study of pelvic adhesions revealed the CD8-positive cells is directly involved in the formation of the immune response at the late stages of adhesiogenesis.
What is known already: One of the reason identifies the high frequency of adhesion formation is the presence of inflammation in the abdominal cavity with different severity and origin. It is known that Insufficiency of the fibrinolytic system, increased levels of a number of cytokines, including transforming growth factor-β1, and tissue hypoxia induce neoangiogenesis and fibrotization of the fibrin matrix, which leads to the formation of adhesions. Data on expression of CD4, CD8, CD20, CD138/syndecan-1 in the pelvic peritoneal adhesions in connection with their prescription, localization and origin is absent at accessible literature.
Study design, size, duration: Two hundred infertile women (aged 19-49 yrs) with pelvic peritoneal adhesions, who were underwent operative laparoscopy and adhesiolysis.
Participants/materials, setting, methods: The material for this study was the fragments of surgical material (adhesions and their parts) n=200, taken from the women of reproductive age who suffered with infertility during operative laparoscopy.The morphological and immunohistochemical study of adhesions were carried out by standard techniques using paraffin blocks, reagents of Dako and monoclonal antibodies to CD4 (Clone 4B12 Ready-to-Use),CD8 (Clone C8/144B Ready-to-Use), CD20 (Clone L26 Ready-to-Use),CD138/syndecan-1 (Clone MI15 Ready-to-Use) of Abcam with automatic coloring Dako Cytomation.
Main results and the role of chance: To assess the population composition of these cell infiltrates, as well as individual diffusely located inflammatory cells, an immunohistochemical method with the main lymphocytic markers (CD4, CD8, CD20, CD138) was used. First of all, it is necessary to note the complete absence of CD20-positively colored cells in all observations, which indicates that at the final stage of the formation of adhesions, there is no element of the B-lymphocytic immune response. In an immunohistochemical study with syndecan-1 (CD138) antibodies, we identified a small number of positively colored cells that were located mainly perivascular, as part of mononuclear infiltrates. Quantitative analysis showed that the number of such cells is 0.8±0.2. When studying CD4–positive T-lymphocytes, it was found that they are usually located in the form of band-shaped infiltrates and focal perivascular clusters. The number of CD4-positive cells in the spike tissue is 5.6±0.2. CD8-positive cells were located mainly submesothelial, and in the form of perivascular clusters, the number of such cells was 9.2±0.6.
Limitations, reasons for caution: Age limitation, only women aged 19-49 yrs took part in this study. Exclusion criteria were the following for the groups: acute gynecological diseases, malignant diseases of female genitalia and ovarian tumors.
Wider implications of the findings: The absence of B-cells in the "mature" adhesions' tissue was found.The number of CD8-positive cells in our study was 1.5 times higher than the number of CD4-positive T-lymphocytes.CD4-positive T-lymphocytes play an important role and their number significantly prevails over the number of CD8-positive T-lymphocytes at the initial stages of adhesiogenesis.
Trial registration number: Case control study

P-745 The efficacy of Buscopan® in reducing pain during ultrasound-guided manual vacuum aspiration (USG-MVA): A double-blind randomised placebo-controlled trial

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Study question: Does Buscopan® reduce abdominal pain experienced by women undergoing ultrasound-guided manual vacuum aspiration (USG-MVA)?
Summary answer: The addition of 20mg Buscopan® intravenous injection was not associated with a statistical reduction in pain score but leads to a higher patient satisfaction score.
What is known already: Ultrasound-guided Manual Vacuum aspiration is a feasible and effective out-patient treatment option for treating early pregnancy loss. However, it is associated with a moderate amount of pain due to uterine contraction.
Study design, size, duration: This randomised, double-blinded, placebo-controlled trial was conducted in a university-affiliated tertiary hospital. The study assessed whether 1 ml of 20mg Buscopan® intravenous injection 5 minutes before the USG-MVA will reduce the abdominal pain experienced by the women immediately and 2 hours after the procedure. Participants were randomised between June 2018 to January 2020 using a computer-generated number series in a 1:1 ratio.
Participants/materials, setting, methods: Women aged 18 years or older with first-trimester miscarriage undergoing the USG-MVA procedure were eligible. In total, 122 participants out of 128 eligible were included. Of whom, 111 underwent the USG-MVA procedure, 60 randomised to the Buscopan® group, and 62 to the placebo group.

Main results and the role of chance: The median abdominal pain scores in the Buscopan® group were 16.0% and 21.2% lower than the placebo group immediately post-procedure and 2 hours after the procedure in the Buscopan® group. Repeated measures ANOVA indicated that the both vaginal and abdominal pain scores improved significantly with the time (Vaginal $F(1,108)=180.1, p<0.0001$; Abdominal: $F(1,108)=83.41, p<0.001$) but not with group. No difference was noted in the complications and side effects profile. The physiological stress measured by Log10 sAA levels reduced significantly with time ($F(2.8,286.1)= 6.3$, $p<0.001$) but not with group ($F=0.1$, $p=0.96$). Women randomised to Buscopan® had a significantly higher ($p=0.032$) mean VAS satisfaction scores compared to those receiving placebo (79.0±17.3 vs 73.4±24.1).
Limitations, reasons for caution: This study was a single-centre study, thus one should be cautious in the overall generalisability of the results.
Wider implications of the findings: Few studies have evaluated the use of anti-spasmodic agents to minimise uterine contraction pain in women undergoing outpatient uterine evacuation. We consider Buscopan® a useful adjunct in the pain control of USG-MVA to specifically reduce uterine cramps. Further larger studies are required to evaluate its efficacy.
Trial registration number: ChiCTR1800014590

P-746 Obstetric outcomes of singleton birth after hysteroscopic division of septate uterus

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Study question: The aim of this study is to determine the obstetric outcomes in patients with a singleton birth after hysteroscopic division of septate uterus.
Summary answer: The data suggest excellent obstetric outcomes for singleton gestation after hysteroscopic division of a septate uterus reaching either the internal or the external cervical os.
What is known already: Septate uterus is a rare Müllerian anomaly with major impact on reproductive outcomes, particularly with a septum over 10mm. Controversy still exists over the need for surgical correction of the septum due to conflicting data on outcomes, particularly in women with histories of good obstetric outcomes and incidental septum findings. Placental location in relation to the septum may account for such conflicting reports. Most data on reproductive outcomes after hysteroscopic surgical correction combine both septate and subseptate uteri. There is limited published data on obstetric outcomes after hysteroscopic surgical correction of septate uteri, especially septate uteri reaching the external os.
Study design, size, duration: This retrospective cohort study included 107 patients with infertility and/or recurrent pregnancy loss (RPL) who received treatment between 2002 -2019. The study group included 24 patients with a singleton birth after hysteroscopic correction of septate uterus (Class Va; ASRM classification) that was diagnosed on trans-vaginal 3D ultrasound. The control group included 83 patients with a singleton birth who had normal endometrial cavity on hysteroscopy during the same period of time, before starting treatment.
Participants/materials, setting, methods: This study was conducted at an infertility clinic affiliated with a teaching hospital. In the study group the septum reached the internal or the external cervical os in 14 and 10 patients respectively. After hysteroscopic correction, all patients were offered various infertility treatments depending on the underlying etiology. The inclusion criterion in this study was to have a singleton birth after hysteroscopy. Demographic and clinical data and obstetric outcomes were compared between the two groups.
Main results and the role of chance: There was no significant difference in mean age, infertility duration, infertility type and incidence of male infertility or ovulatory disorders between the two groups. There was a significantly higher BMI (0.048), and a higher incidence of history of miscarriage ($P=0.002$) and history of RPL ($P=0.017$) in the study group. There was significant lower incidence of tubal factors infertility ($P=0.005$) and endometriosis ($P=0.03$) in the study group, therefore there was higher incidence of spontaneous conception (70.8% vs 19.3%; $P=0.000$) and lower incidence of conception with IVF-ET (20.8% vs 66.3%; $P=0.000$) in the study group compared to the control group respectively. There was significantly higher incidence of prophylactic cervical cerclage (17.4% vs 0%; $P=0.000$), and delivery by CS (69.6% vs 41.2%; $P=0.019$) and lower incidence of vaginal delivery (30.4% vs 58.8%; $P=0.019$), in the study group compared to the control group. There was no significant difference in gestational age in weeks (38.3+1.8 vs 38.6+2.0), newborn birth weight in grams (3173.9+630.0 vs 3202.1+555.6), incidence of premature birth (12.5% vs 12.2%), or other obstetric complications (25% vs 17.6%) between the study and the control groups respectively. For premature births, mean gestational age was 34.3+0.47 and 34.6+1.2 weeks in the study and control groups respectively.
Limitations, reasons for caution: A retrospective study has its own inherent bias. Furthermore, the small sample size is explained by the fact that a septate uterus is a rare anomaly leading to difficulties finding cases and organizing a prospective study to achieve a larger sample size. A multicenter prospective study is needed.
Wider implications of the findings: Regardless of whether the septum reached the internal or external os, there were excellent obstetric outcomes in singleton gestations after hysteroscopic correction of septate uteri. There was no increased risk with septate uteri involving the cervix. Hysteroscopic surgical correction should be the treatment of choice for patients with septate uteri.
Trial registration number: Not Applicable

P-747 Implementation of the ESHRE Congenital uterine anomaly classification into practice and clinical pregnancy outcomes at a Tertiary University Teaching Hospital Fertility department

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Study question: What's the incidence of class U1-U6 CUAs in subfertile women? What's the clinical pregnancy rate in women with the most common anomaly, a septate uterus?
Summary answer: The incidence of CUAs is 5.9% in our subfertile population, with a septate uterus (U2) being the most common abnormality in 4.2% of the population
What is known already: Congenital uterine anomalies (CUAs) are common. A systematic review suggested an estimated overall prevalence of 5.5% in an unselected population, 8.8% in the subfertile population, 13.3% in those with a history of recurrent miscarriage and 24.5% in those with a history of subfertility and recurrent miscarriage. A septate uterus (U2) is the most common CUA and is amenable to surgical intervention although at present there is a lack of evidence suggesting benefit in subfertile patients. Women with a septate uterus are known to have poorer reproductive outcomes, including reduced conception rate and increased first trimester loss.
Study design, size, duration: All patients referred to our department for subfertility had a 2D pelvic ultrasound scan as part of their baseline investigations. Since it was established in 2016, all patients with a suspected CUA based on clinical history and investigations, were referred to the clinic and data collected prospectively. Prior to this, women with suspected CUAs required a hysteroscopy or MRI scan for confirmation of diagnosis, often leading to long waiting lists and treatment delays.
Participants/materials, setting, methods: Out of the 4716 patients referred to the department for subfertility from 2016-2018, 302 women were referred to the 3D clinic due to suspicion of a CUA. Transvaginal 3D-ultrasound scan was performed and CUAs classified according to the ESHRE/ESGE working groups. Patients diagnosed with a septate uterus were given options of conservative versus surgical treatment, in the light of unclear benefits of hysteroscopic septum resection. Clinical pregnancy data were collected about this cohort.
Main results and the role of chance: Of the 302 women referred to the service, the uteri of 25 patients were unable to be assessed accurately, most commonly as the cavity was unclear due to a thin endometrium. The remaining

277 patients were classified as having the following CUAs; Normal (U0) 63 patients, Dysmorphic (U1) 5 patients, Septate (U2) 199 patients, Bicorporeal (U3) 6 patients and Hemi uterus (U4) 4 patients. No women were classified as having an aplastic uterus (U5) or unclassified (U6).

Of the 199 women with a septate uterus, 15 women opted for surgical intervention, 143 women decided to have conservative management and 41 women were lost to follow up. The women who had hysteroscopic resection of the septum had a mean age of 35 years, 6/15 had primary subfertility and 6/15 had a history of recurrent miscarriage. The women who had conservative management had a mean age of 32.5 years, 100/143 had primary subfertility and 20/143 had a history of recurrent miscarriage. At present, 89/143 women who have had conservative management and 12/15 women who had surgical interventions have had a clinical pregnancy, 72/89 and 6/12 of these pregnancies were IVF/ICSI pregnancies respectively.

Limitations, reasons for caution: This is an observational study, these findings can be useful for patient counselling. However, ideally randomised controlled trials are needed as evidence for the different treatment options for the cohort of patients with septate uterus, which are largely lacking in the current literature, as their feasibility remains a challenge.

Wider implications of the findings: Three-dimensional transvaginal ultrasonography clinics are cost-effective one-stop services, successfully providing a diagnosis and management plan in 92% of patients referred with a suspected CUA. They increase patient satisfaction by providing an opportunity to discuss risks in future pregnancies and reducing reliance on hysteroscopy and MRI scans.

Trial registration number: Not Applicable

P-748 Diode laser hysteroscopic metroplasty for dysmorphic uterus: a pilot study

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Study question: Whether diode laser hysteroscopic metroplasty for dysmorphic uterus is a safe and efficacious procedure and its effect on reproductive outcomes.

Summary answer: Diode laser hysteroscopic metroplasty is a safe and effective procedure for infertile women with dysmorphic uterus with comparable results to those reported in the literature.

What is known already: A T-shaped uterine anomaly is categorized by the ESHRE/ESGE consensus as dysmorphic uterus class U1a, characterized by an abnormal hypoplastic uterine cavity. A Y-shaped uterus is a dysmorphic uterus with a fundal subseptum. Dysmorphic uteri are associated with infertility, recurrent implantation failure (RIF), recurrent pregnancy loss (RPL), and adverse pregnancy outcomes. According to several studies, it seems that hysteroscopic metroplasty may improve the chances of conception and live birth. Previous studies described the procedure using bipolar systems, monopolar needle or scissors. The purpose is to achieve a uterine cavity of normal shape and volume by cutting the thickened lateral walls.

Study design, size, duration: This was a retrospective pilot study with a prospective follow-up. We retrospectively evaluated all cases operated between February 2018 to February 2020, at Hillel Yaffe Medical Center, Hadera, Israel. Reproductive outcomes for women who underwent the procedure were followed until September 2020. Pregnancies that were ongoing on September 2020 were followed until January 31st 2021.

Participants/materials, setting, methods: Nulliparous women with a diagnosis of infertility or RPL, who were diagnosed with dysmorphic uterus by three-dimensional ultrasound (3D-US) and underwent diode laser hysteroscopic metroplasty were included. All the metroplasties were done in one tertiary center by the same specialist. Reproductive outcomes were evaluated retrospectively and prospectively for a total follow-up time of 32 months. Reproductive performances before and after metroplasty were compared where possible.

Main results and the role of chance: Twenty-five women underwent diode laser hysteroscopic metroplasty for dysmorphic uterus in our institute. No perforations, excessive bleeding, or other complications were encountered during the procedures. Follow-up hysteroscopy and 3D-US were satisfactory in all cases 2 months after the metroplasty. A total of 15 nulliparous women returned to fertility treatments afterwards, among whom 9 conceived (60% pregnancy rate). Their infertility period before the procedure was 56.6 ± 36.1 months. The duration between the metroplasty to pregnancy was 5.2 ± 3.5 months. The rate of deliveries and ongoing pregnancies (pregnancies beyond 20 weeks of gestation) was 78% (7/9), with five successful liveborn deliveries and two ongoing pregnancies. All deliveries were between 36-37 weeks. The 10 women who were not treated by our infertility unit were contacted, among whom 6 discontinued their attempt to conceive. The other 4 conceived; three of them spontaneously. Among those 4 women,the rate of deliveries and ongoing pregnancies was 75%, with one term delivery and two ongoing pregnancies.

Limitations, reasons for caution: First, we included both T-shaped and Y-shaped uteri as both represent close versions of dysmorphic uteri, but in fact they differ. The subseptum might interfere with reproduction in a different mechanism. Second, the small and heterogeneous sample as well as the short duration of follow-up limit the conclusions.

Wider implications of the findings: We present the first application of diode laser in hysteroscopic metroplasty for dysmorphic uteri. This technique seems promising and our results are comparable with other series using different cutting devices. Only larger controlled trials with a longer follow-up can confirm the safety, efficacy, and impact on reproductive outcomes

Trial registration number: Not Applicable

P-749 Knowledge of women undergoing surgery for endometrioma regarding the impact of the disease and its treatment on ovarian reserve and fertility

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Study question: Do women undergoing surgery for endometrioma due to pain, a cyst and/or subfertility understand the impact of the disease and its treatment on ovarian reserve and fertility.

Summary answer: The majority of women treated in a fertility setting are well informed compared to those in a general medical setting. What is known already: Infertility affects 30% to 50% of women with endometriosis. Ovarian endometriomas are reported in 17-44% of infertile women with endometriosis and are typically associated with more severe disease. Endometriomas are associated with diminished ovarian reserve, due to the endometrioma per se or due to surgical interventions required to treat and excise the disease. ESHRE guidelines recommend that women should be informed pre-operatively of the potential reduction in ovarian reserve associated with surgery and that ovarian reserve tests should be performed when future fertility is a concern.

Study design, size, duration: In conjunction with our histopathology colleagues we identified a cohort of women with a histological diagnosis of one or more ovarian endometriomas who underwent surgery in our unit between 2010 and 2019. We developed a scoping questionnaire, targeted at women currently over the age of 40, who had previously undergone surgery for endometrioma under the age of 35. Patients were contacted by telephone and consent obtained to send an email with a survey link.

Participants/materials, setting, methods: We identified 47 women who had a histological diagnosis of endometrioma. Of these, 30 were contactable by telephone, of whom 29 consented to being sent information regarding the study and a link to the questionnaire. 21 women completed the survey. Respondents were divided into 2 groups for analysis. Group 1 cited 'fertility' or 'both pain and fertility' as an indication for their surgery while group 2 had 'pain' or 'ovarian cysts' but no fertility concerns. Main results and the role of chance: The majority (62%) of patients were diagnosed with endometriosis while aged 25-34. The indication for surgery was evenly divided between pain (32%), fertility (37%) and ovarian cysts (37%). 60% of women reported having endometriomas diagnosed preoperatively. Striking differences were noted between groups 1 and

2. Of the women who cited 'fertility' or 'both pain and fertility' (n=9) as an indication for their surgery, 78% (n=7) reported being aware of any possible negative impact of endometriosis on their fertility, with 78% also being aware of the possible negative impact of surgery for endometriosis on their fertility. This compared to only 36% (n=4) and 27% (n=3) respectively in Group 2. In group 1, 56% (n= 5) remembered having an AMH level checked pre operatively while 78% (n=7) also had an ultrasound pre-operatively.

In contrast, only 33% (n=3) of Group 2 remember having an AMH level checked pre operatively though 64% (n=7) had an ultrasound pre-operatively.

Of those whose surgery was performed by a fertility specialist, 75% (n=6) reported being aware of the impact of endometriosis and also the impact of surgery on ovarian reserve, compared to 44% (n=4) of those who surgery was performed by a non-fertility specialist.

Limitations, reasons for caution: This is a retrospective study and the numbers are small. We were only able to identify women with an endometrioma via pathology records, so those with no excision of disease (eg those who had ablation of an endometrioma) were excluded from this analysis.

Wider implications of the findings: This suggests the majority of patients treated in a fertility setting are counselled regarding the benefit of surgery but also the risk to ovarian reserve. This is not the case in other settings. It is time to disseminate guidelines such as those produced by ESHRE to our general gynaecology colleagues.

Trial registration number: not applicable

P-750 Clinical efficacy of virtual reality for acute pain and anxiety management during outpatient hysteroscopy and endometrial biopsy in subfertile patients

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Study question: Does the use of virtual reality (VR) headsets in diagnostic office hysteroscopy (HSC) with endometrial biopsy (EB) reduce anxiety and pain scores in the patient?

Summary answer: Virtual reality during office HSC do not seem to improve relaxation, anxiety, or pain scores. Physicians have a good perception of patients' pain.

What is known already: Women undergoing outpatient HSC experience high levels of preoperative anxiety, which increase pain and discomfort during the procedure. The experience of pain is a complex phenomenon, which simultaneously occurs on cognitive, emotional, and behavioural levels, and is influenced by many factors. A Cochrane review failed to show a significant difference between different types of pain relief (analgesics, local anaesthetic and verbal support techniques …). VR is a multisensory immersion providing an interactive high level distraction, occupying a large portion of humans' finite attentional resources (vision and audio), and leaving less cognitive capacity available to process pain.

Study design, size, duration: The sample size for this prospective randomized controlled trial was calculated at 196 patients (98 per group), considering a power of at least 80% to detect superiority of adding a VR headset versus standard care, standard deviation (SD=2.0), using a two-sided, t-test, at significance level alpha of 0.05.

The preliminary results after 1 month include a sample of 48 patients: 25 in the VR group and 23 controls.

Participants/materials, setting, methods: All 48 patients suffer subfertility and underwent HSC with EB at our tertiary-care fertility center. We used Oncomfort®, a commercially available VR autohypnosis relaxation program designed for perioperative settings. The headmounted smartphone display with headphones provides image sound distraction with suggestive hypnosis techniques incorporated. Before and immediately after the exam, both patients and surgeons fill out a questionnaire using the 10.0cm visual analog scale (VAS).

Main results and the role of chance: The mean duration of HSC was 3min43sec in the VR group, (range 2-6min), compared to 4min50 in the control group (range 1-12minutes), which was not significantly different (p=0.09). Subjective variables of stress, anxiety and pain were evaluated at four different time points, i.e. before, during, immediately after HSC and one week later.

According to VAS, stress levels did not differ significantly (p>0.05) between the VR group and the control group, or within time: 5.08 to 5.36 to 3.08 vs 4.48 to 4.83 to 2.48 before, during and after HSC respectively. Fear levels prior to HSC at 4.28 for VR patients and 3.52 for controls did not increase significantly during HSC in both groups: 4.44 vs 4.17. During HSC, pain levels increased from 1.40 to 4.720 in the VR group vs 0.65 to 4.109 (NS) in the controls, to decrease again afterwards to 2.60 vs 2.17 (NS) respectively.

Physicians rated the average pain levels of VR patients as 3.32 compared to 3.0 for controls, which was significantly correlated to patients' perception (p<0,005). Patients gave a positive rating to the VR experience (satisfaction score 7.17).

Limitations, reasons for caution: These are preliminary results, evaluating only a fourth of the required sample. A population selection bias could exist, as recruited patients were willing to accept VR. The very short induction period of 2 minutes could influence the effect of (immersiveness into) VR.

Wider implications of the findings: Pain management in ambulatory procedures should be multimodal and should include both pharmacological and non-pharmacological interventions. Introducing VR might increase patient tolerance for longer or more painful procedures. Offering a range of options will increase the spectrum of successful procedures *in the outpatient setting* and improve patient experience.

Trial registration number: B.U.N. 1432020000050

POSTER VIEWING
SAFETY AND QUALITY OF ART THERAPIES

P-751 Immediate versus postponed frozen-thawed embryo transfer after IVF/ICSI: a systematic review and meta-analysis

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Study question: Can frozen embryo transfer (FET) be offered immediately after a stimulated IVF/ICSI cycle without compromising live birth rate (LBR)?

Summary answer: FET in the menstrual cycle immediately following the stimulated IVF/ICSI cycle was associated with a slightly higher LBR compared to standard postponed FET.

What is known already: It is standard clinical practice to postpone FET for at least one menstrual cycle following a failed fresh transfer or a freeze-all cycle. This practice is thought to minimize any possible residual negative effect of ovarian stimulation, with excessive steroid levels and multiple corpora lutea, on the resumption of a normal ovulatory cycle and receptivity of the endometrium. Even so, elective deferral of FET is an empirical strategy based on suggestions rather than solid scientific evidence and may unnecessarily delay time to pregnancy, causing frustration and decreased quality of life to couples.

Study design, size, duration: Systematic review and meta-analysis according to PRISMA guidelines. Original studies on subfertile women aged 18-46 with any indication for treatment with IVF/ICSI investigating the timing of FET after IVF/ICSI were included. Intervention was defined as FET in the menstrual cycle immediately following the stimulated IVF/ICSI cycle. Comparator was defined as FET in the second or subsequent menstrual cycle following IVF/ICSI. Risk of bias was assessed using Robins-I and quality of evidence using GRADE.

Participants/materials, setting, methods: PubMed (MEDLINE) and EMBASE databases were searched for MeSH and Emtree terms, as well as text words related to timing of FET, up to March 2020. There were no limitations regarding year of publication or duration of follow-up but to English language. The primary outcome was LBR. Secondary outcomes were implantation rate, pregnancy rate, clinical pregnancy rate (CPR), time-to-pregnancy, miscarriage rate (MR), cycle cancellation rate and patient wellbeing.

Main results and the role of chance: Out of 4124 search results, 15 studies were included in the review. Studies reporting adjusted odds ratios (aOR) for

LBR, CPR and MR were included in meta-analyses. All studies (n=15) were retrospective cohort studies involving a total of 6,304 immediate FET cycles and 13,851 postponed FET cycles including 8,019 matched controls. Twelve studies of very low to moderate quality reported no difference in LBR with immediate versus postponed FET. Two studies of moderate quality reported a statistically significant increase in LBR with immediate FET and one small study of very low quality reported better LBR with postponed FET. Trends in rates of secondary outcomes followed trends in LBR regarding timing of FET. The meta-analyses showed a significant advantage of immediate FET (n=2,076) compared to postponed FET (n=3,833), with a pooled aOR of 1.20 (95% CI 1.01-1.44) for LBR and a pooled aOR of 1.22 (95% CI 1.07-1.39) for CPR. Limitations, reasons for caution: Limitations include the retrospective design and heterogeneity of studies included, limiting comparison and pooling of data. With little transparency regarding cancellation rates, the risk of selection bias is apparent. Further, confounding by embryo quality is a limitation. Small sample sizes are a limitation to subgroup meta-analyses.

Wider implications of the findings: The standard clinical practice of postponing FET for at least one menstrual cycle following a failed fresh transfer or a freeze-all cycle may not be best clinical practice. Randomized controlled trials including data on cancellation rates are highly needed to provide high grade evidence regarding clinical practice and patient counseling.

Trial registration number: not applicable

P-752 Embryo morphological grading across several IVF centers is not consistent but an interactive training is useful to improve its consistency

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Study question: Are the embryologists across several IVF clinics concordant when evaluating embryo morphology?

Summary answer: Embryo morphological grading is sufficiently consistent among embryologists from the same center, while an interactive training was essential to improve its accuracy across several clinics.

What is known already: Embryo morphology, mostly at the blastocyst stage, is the strongest non-invasive embryological feature that associates with implantation potential. This association is confirmed also when euploid blastocysts are transferred. At present, several embryo grading schemes exist but is still unclear which is the most effective among them. Moreover, many IVF clinics adopt internal embryo grading scores, further limiting the transferability of this crucial prognostic information across different laboratories. With the aim of assessing the level of concordance in embryo grading within and between IVF clinics, the Italian Society of Embryology, Reproduction and Research (SIERR) conceived this study.

Study design, size, duration: We photographed 40 cleavage-stage and 40 blastocyst-stage embryos (3 focal-planes=240 photos). Two embryologists (senior and junior) from 65 Italian IVF clinics were invited to grade them. Their evaluations were blindly collected as Phase-I (January2020-March2020). Phase-II consisted of an interactive-training on Google-Classroom during which 6 selected experts found a Consensus on the morphological evaluation of the 80 embryos (April2020). As Phase III (May2020-July2020), a second set of 240 pictures was sent to senior participants and experts.

Participants/materials, setting, methods: Eighteen centers agreed to participate, and 36 embryologists were included. The embryo grading scheme adopted was the Alpha-ESHRE Istanbul Consensus (parameters: cleavage-stage blastomeres' symmetry and fragmentation, blastocyst's expansion, inner-cell-mass and trophectoderm quality), conventionally used in 50% of the centers (N=9/18). The concordance within (junior versus senior) and between (senior versus experts) centers was calculated through the Cohen's-k. The concordance between centers was compared before and after the interactive training on the two sets of pictures.

Main results and the role of chance: The centers and embryologists included were representative of the Italian IVF scenario: oocyte-retrievals per year:711±636,range100-2200; cycles with cleavage-stage embryo-transfer:322±339,0-1300; cycles with blastocyst-stage embryo-transfer:390±403,0-1100; operators per center:5.6±4.0,2-13; senior embryologists' experience:14.8±7.4yr,7-30; junior embryologists' experience:2.7±0.6yr,1-3. The intra-center concordance was (i)for blastomeres' symmetry 82±15% (38-100%), k 0.59±0.27 (0.02-1), (ii)for blastomeres' fragmentation 88±9% (65-100%), k 0.71±0.2 (0.29-1), (iii)for blastocysts' expansion 80±16% (48-100%), k 0.66±0.27 (0.19-1), (iv)for inner-cell-mass quality 73±16% (35-95%), k 0.58±0.24 (0.07-0.92), (v)for trophectoderm quality 71±19% (38-95%), k 0.54±0.32 (0.01-0.97). Linear regressions showed no association of centers' and embryologists' characteristics with all k-values.

Among clinics with the highest mean number of cycles per year and intra-center concordance, we selected 6 experts for the interactive-training. We then calculated the inter-center concordance as the agreement rate between senior embryologists and the experts for phase-I and phase-III: (i)for blastomeres' symmetry 67±15% (30-85%) and 73±17% (15-90%;Wilcoxon-signed-ranks-test=0.06), k 0.33±0.22 (-0.29-0.58) and 0.42±0.33 (-0.56-0.77); (ii)for blastomeres' fragmentation 81±17% (23-95%) and 83±14% (50-95%;Wilcoxon-signed-ranks-test=0.8), k 0.54±0.22 (-0.05-0.84) and 0.55±0.22 (0.17-0.81); (iii)for blastocysts' expansion 59±16% (35-85%) and 67±17% (23-90%;Wilcoxon-signed-ranks-test=0.04), k 0.35±0.20 (0.06-0.73) and 0.44±0.22 (-0.10-0.7); (iv)for inner-cell-mass quality 60±14% (33-80%) and 69±11% (48-85%;Wilcoxon-signed-ranks-test=0.02), k 0.40±0.20 (0.01-0.69) and 0.51±0.18 (0.18-0.77); (v)for trophectoderm quality 55±12% (23-70%) and 63±10% 48-78%;Wilcoxon-signed-ranks-test<0.01), k 0.29±0.15 (-0.08-0.52) and 0.42±0.15 (0.21-0.66).

Limitations, reasons for caution: Only 28% (N=18/65) of the Italian IVF centers invited to participate responded to the survey. The conventional adoption of grading schemes other than Istanbul-Consensus by 50% of the embryologists might have biased their evaluation. The experts were not fully-concordant in grading 13.8% of the embryos (N=22/160), which required active discussions.

Wider implications of the findings: Blastocyst-grading concordance was significantly improved after the training phase. Therefore, interactive consensus meetings and training platforms are keenly needed to standardize this practice across the centers. The "avant-garde" of artificial intelligence applied to embryo image analysis might help overcoming this issue in the future.

Trial registration number: N.A.

P-753 A randomised controlled trial comparing expectant management versus intrauterine insemination in couples with unexplained subfertility and a poor prognosis for natural conception

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Study question: Does 6 months expectant management reduces ongoing pregnancy rates compared to intrauterine insemination with ovarian stimulation (IUI-OS) in couples with unexplained subfertility?

Summary answer: In couples with unexplained subfertility and a poor prognosis for natural conception, 6 months of expectant management decreases ongoing pregnancy rates as compared to IUI-OS.
What is known already: In couples with unexplained subfertility and a poor prognosis, IUI-OS is a first line treatment. We have previously shown that in couples with unexplained subfertility and a good prognosis for natural conception (>30% in 12 months), 6 months expectant management does not reduce pregnancy changes. However, in couples with a poor prognosis for natural conception, effectiveness of IUI-OS is uncertain.
Study design, size, duration: We performed a non-inferiority multicentre randomised controlled trial (RCT) within the infrastructure of the Dutch Consortium for Healthcare Evaluation and Research in Obstetrics and Gynaecology. We studied couples with unexplained subfertility and a poor prognosis for natural conception. The couples were allocated in a 1:1 ratio to six months expectant management or six months IUI-OS with either clomiphene citrate or gonadotrophins.
Participants/materials, setting, methods: We intended to include 1091 couples. The trial was halted pre-maturely due to slow inclusion after randomisation of 178 couples. The primary outcome was ongoing pregnancy leading to a live birth with multiple pregnancy and miscarriage rate as important secondary outcomes. We calculated relative risks with 95% CI and a corresponding hazard-rate for ongoing-pregnancy-over-time based on intention-to-treat.
Main results and the role of chance: Between October 2016 and September 2020 92 couples were allocated to expectant management and 86 to IUI-OS. Baseline characteristics were equally distributed. Mean female age was 34 years, median duration of subfertility was 21 months. Within 6 months after randomisation, women allocated to expectant management had a lower ongoing pregnancy rate than women allocated to IUI-OS (12/92 [13.0%] vs 29/86 women [33.7%], risk ratio 0.39 (95%CI 0.21 to 0.71)). There were two ongoing twin pregnancies in the expectant management group versus none in the IUI-OS group. Of 15 clinical pregnancies in the expectant management group three miscarried (20%), of 36 clinical pregnancies in the IUI-OS group seven miscarried (19.4%) (RR 1.03 (95% CI 0.31 to 3.45)). For the outcome ongoing pregnancy, the hazard ratio for expectant management versus IUI-OS was 0.34 (95%CI 0.18 to 0.67).
Limitations, reasons for caution: Our trial did not reach the planned sample size and therefore the results are limited by the number of participants. As 8 women are still pregnant, in this abstract we report ongoing pregnancy rates. Live birth rates will be presented at the conference.
Wider implications of the findings: In couples with unexplained subfertility and a poor prognosis for natural conception, expectant management is inferior to IUI-OS. We advise the basic work-up for subfertility to contain a prognostic assessment, and when subfertility is unexplained and natural fertility prospects are poor IUI-OS should be the preferred treatment.
Trial registration number: NTR5599

P-754 A novel warmed device protecting the embryo transfer catheter is effective in preventing the cooling of embryos during the embryo transfer procedure

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Study question: Can cooling of embryos during the embryo transfer be alleviated with the use of a 37°C temperature protective device covering the ET catheter?
Summary answer: Cooling of embryos during embryo transfer can be effectively alleviated by using a 37°C pre-warmed temperature protective device covering the ET catheter.
What is known already: An optimized physicochemical environment is crucial for maintenance of normal homeostasis, metabolism, and spindle stability to minimize stress on gametes and embryos. During preimplantation embryo development, epigenetic reprogramming occurs and environmental stress factors including temperature can disrupt this critical process and potentially damage embryos. IVF laboratories use heated stages, warming blocks and incubators to control and maintain temperature within set control limits. However, it has recently been shown that during the ET procedure, the temperature of fluid in the catheter tip drops significantly. To date no means of preventing this has been reported, or to our knowledge, implemented.
Study design, size, duration: In this prospective controlled study, 100 simulated embryo transfer procedures were carried out at 5 European clinics. The catheters were loaded with medium according to clinic protocol. In 50, the transfer catheter was then transported to the clinician and handled according to standard practice, and in the other 50 the catheter was covered with the temperature protecting device after loading but otherwise handled identically. 10 control and 10 intervention procedures were performed at each clinic.
Participants/materials, setting, methods: The temperature inside the ET catheter tip (Wallace Sure View) was measured with a calibrated thermocouple probe (diameter of 0.25 mm) placed at the location of an embryo and monitored during standard operating ET procedures (control group), and with the ET catheter-syringe set inserted into a temperature protective device (37°C pre-warmed aluminium core, 15x90 mm) allowing retraction of the ET catheter tip immediately after embryo loading (study group). No embryos were employed in this study.
Main results and the role of chance: During standard operating ET procedures (control group), a considerable variation was observed in the embryo loading temperature between clinics, ranging from 34°C to 37°C. A profound temperature drop down to 20.8°C-25.6°C was recorded within 20 seconds of loading the ET catheter and in all 5 clinics a very rapid decline in catheter tip temperature down to ambient temperature was observed regardless of environment, type of workstation, or standard operating ET procedures in use. In contrast, when the ET catheter-syringe set was placed into a 37°C pre-warmed temperature protective device from the time of embryo loading until the end of the simulated ET procedure, the drop of temperature was minimal, effectively maintaining the temperature at the loading temperature of between 34°C and 37°C °C throughout the simulated ET procedure. The mean loss of temperature of 14.8°C in the control group was reduced to just to 0.4°C in the study group. The consistent and profound differences in catheter tip temperature between the control and device groups across repeated measurements at different sites indicate the findings to be robust.
Limitations, reasons for caution: Numerous permutations of laboratory culture systems exist and the equipment, consumables and procedure for ET, including time, are highly variable and operator dependent. Therefore, the results and conclusions of this study may not be universally applicable. Furthermore, the impact of embryo cooling during ET on live birth rate remains uncertain.
Wider implications of the findings: The ET procedure represents a 'weak link' in temperature control from the IVF laboratory to the patient until the embryo is safely deposited into its physiological environment, the uterine cavity. We demonstrate the effectiveness of a novel device for maintaining the temperature during ET, which could potentially improve embryo viability.
Trial registration number: not applicable

P-755 Perinatal outcomes following Day-4 embryo transfer compared to Day-2, Day-3 and Day-5 embryo transfer: an analysis of 56,346 singleton live births

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Study question: Are perinatal outcomes of singleton live births following Day-4 embryo transfer (ET) different to Day-2, Day-3 and Day-5 ET?
Summary answer: Perinatal outcomes of singleton live births following Day-4 ET are similar with those following Day-2, Day-3 and Day-5 ET.
What is known already: The morula represents a critical stage in preimplantation embryo development, but the usage of morula transfer on Day-4

has received little attention. Recent work from our group suggested that live birth rates following Day-4 ET appear higher than cleavage-stage ET, but lower than blastocyst ET. Therefore, Day-4 appears an alternative day to perform ET, offering the benefits of extended culture for embryo selection, but with shorter in-vitro culture exposure, as well as improving flexibility and planning in the IVF Clinic. However, there are extremely limited data available on the perinatal outcomes after Day-4 ET compared to cleavage-stage and blastocyst ET.

Study design, size, duration: Retrospective cohort study using data from the anonymised dataset of the Human Fertilisation and Embryology Authority (HFEA) in the UK between 2000 and 2016. Data from singleton live births of women undergoing their first IVF/ICSI cycle were analysed to compare perinatal outcomes after fresh Day-2,3,4,5 embryo transfers.

Participants/materials, setting, methods: Births resulting from the first, fresh, autologous, stimulated, non-PGT cycles, with full data, were included. After exclusions, a total 56,346 singleton live births were included in the analysis (17,613 from Day-2 ET, 15,533 from Day-3 ET, 508 from Day-4, 22,692 from Day-5 ET).

Binary/multinomial logistic regression analysis was performed to adjust for important cofounders. Adjusted odds ratios (aORs) and 95% confidence intervals (95%CI) were calculated. The level of significance was set at <0.05.

Main results and the role of chance: The probabilities of birth at full-term (FT) and normal birthweight (NBW) after Day-4 transfer (FT 90.4%; NBW 84.6%) were similar to Day-2 (FT 89.7%, aOR 0.994, [0.734-1.344]; NBW 81.9%, aOR 0.881, [0.708-1.096]), Day-3 (FT 90.2%, aOR 1.026, [0.760-1.386]; NBW 82.4%, aOR 0.894, [0.719-1.111]) and Day-5 transfer (FT 90.4%, aOR 1.001, [0.743-1.350]; NBW 83.7%, aOR 0.920, [0.741-1.142]).

The probabilities of preterm birth (PTB) and very preterm birth (VPTB) after Day-4 transfer (PTB 9.3%; VPTB 0.4%) were similar to Day-2 (PTB 9.5%; aOR=0.952; VPTB 0.8%; aOR=2.172), Day-3 (PTB 9.0%, aOR=0.920; VPTB 0.9%, aOR=2.174), and Day-5 transfer (PTB 8.8%; aOR=0.955; VPTB 0.8%, aOR=1.956).

The probabilities of very-low birthweight (VLBW), low birthweight (LBW), high birthweight (HBW) and very-high birthweight (VHBW) after Day-4 transfer (VLBW 0.9%, LBW 7.9%, HBW 6.3%, VHBW 0.3%) were similar to Day-2 (VLBW 1.8%, aOR=1.827; LBW 8.0%, aOR=1.015; HBW 8.1%, aOR=1.174; VHBW 0.2%, aOR=0.590), Day-3 (VLBW 1.8%, aOR=1.788; LBW 7.4%, aOR=0.927; HBW 8.3%, aOR=1.256; VHBW 0.2%, aOR=0.503) and Day-5 transfer (VLBW 1.6%, aOR=1.782; LBW 6.9%, aOR=0.894; HBW 7.5%, aOR=1.215; VHBW 0.2%, aOR=0.796).

The probability of having a female baby after Day-4 transfer (51.6%) was similar to Day-2 (49.2%, aOR 0.940), Day-3 (49.3%, aOR 0.931) and Day-5 transfer (48.3%, aOR 0.869).

Limitations, reasons for caution: The study is limited by its retrospective nature, the inability to adjust for additional confounders and the small number of singleton births after Day-4 ET. It is not known how Day-4 ET was decided. The incidence of congenital abnormalities was not analysed due to incomplete registration in the dataset.

Wider implications of the findings: Perinatal outcomes of singleton live births following Day-4 ET are similar with those following Day-2, Day-3 and Day-5 ET, suggesting that morula transfer is equally safe as cleavage-stage and blastocyst transfer. Data on a larger number of live births from well-designed RCTs are required to confirm these findings.

Trial registration number: not applicable

P-756 Predictive factors influencing multiple live births in cumulative IVF cycles: retrospective analysis of over 265000 embryo transfer procedures from the national French registry

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Study question: What are the factors that could predict the number of embryos to be transferred in order to diminish risk of multiple pregnancies?

Summary answer: Single embryo transfer (SET) is advisable for <38 year-old women in fresh cycles and for <35 year-old women in FET whatever the IVF number attempts.

What is known already: Multiple pregnancies are associated to increased maternal and perinatal complications. Risks associated to multiple implantations are significantly reduced with SET policy. However, while SET is more assertive with a lesser negative impact in younger patients (<35 years), its feasibility is less evident for the older population, whom oocyte quality is likely compromised. A double embryo transfer (DET) could improve chances of implantation and shorten their time to pregnancy. Identification of risk factors for multiple pregnancies could help in decision making for a double or SET and reduce chances for multiple gestations without reducing the chances to achieve pregnancy.

Study design, size, duration: A retrospective study from the national French data registry provided and approved by the Agence de la Biomédecine was performed. A total of 196530 fresh and 68913 frozen cycles from women aged 18-43 year-old were included (2014-2017). Risk factors assessed included women's age, number of attempts, number of oocytes, fertilization rate, embryo stage, number of embryos transferred, number of supernumerary embryos frozen. Secondary infertility, oocyte donor, oocyte freezing, PGT, freeze-all and IVM cycles were excluded.

Participants/materials, setting, methods: Cumulative cycles derived from 65% of ICSI, 32% of IVF and 3,2% IVF/ICSI. The distribution of patients age at oocyte retrieval was 60% < 35, 21% < 38, 11% < 40, and 8% ≥ 40 years old. Multivariable logistic regression was conducted to calculate adjusted odds ratios with 95% confidence intervals for live birth chance and multiple live birth risk associated with each risk factor.

Main results and the role of chance: The chances of obtaining a cumulative live birth decreases with increased patients age (OR 0.71 for 35-38 years and 0.47 for 38-40 years, $p<0.00001$), with increased number of attempts (from OR 0.87 for attempt = 2 to OR 0.74 for attempt ≥ 4, $p<0.00001$), and for frozen embryos transferred (OR 0.14, $p<0.00001$). The chances of live birth increases with the increased number of oocytes (from OR 1.33 for 4-12 to OR 1.52 for > 18, $p<0.00001$ in all cases), with a fertilisation rate >40% (OR 1.29, $p<0.00001$), with blastocyst transfer (OR 1.29, $p<0.00001$), with the increase on the number of frozen embryos (OR 7.37 for >1, OR 13.08 for >2, and OR 16.92 for >6, $p<0.00001$ in all cases) and number of embryos transferred (OR 1.42 for 2 embryos and OR 1.39 for >2 embryos, $p<0.00001$ in all cases).

In case of live birth, the risks of multiple births when two embryos were transferred decreases in patients aged >38 years (OR 0.50, $p<0.00001$) and for frozen embryos transferred (OR 0.65, $p<0.00001$). The risk increases with a fertilisation rate >60% (OR 1.30, $p<0.00001$), with blastocysts transfer (OR 1.34, $p<0.00001$) and when at least one supernumerary embryo is frozen (OR>1.30, $p<0.00001$).

Limitations, reasons for caution: This study is limited in only providing a risk-benefit balance for multiples on the choice of transferring one or two embryos. Clinical data such as stimulation protocols and doses of gonadotropins were not considered in this evaluation.

Wider implications of the findings: This study provides help to develop a strategy for the medical staff in the decision making for the number of embryos to be transferred. It may also serve as a patient's information aid and help to improve their chances of achieving a health singleton if pregnant.

Trial registration number: not applicable

P-757 The risk of aspirin and prednisone using in women with antithyroid antibodies undergoing assisted reproductive technology

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Study question: Is it safe using aspirin (A) and prednisone (P) before pregnancy among women with antithyroid antibodies (ATAbs) undergoing assisted reproductive technology?
Summary answer: Combination therapy of aspirin and prednisone didn't improve likelihood of clinical pregnancy, but increased miscarriage rate.
What is known already: Compared with women with negative-ATAb, women with positive-ATAb had a lower live birth rate and a higher miscarriage rate. Insufficient evidence existed to determine whether aspirin and prednisone therapy improved the success of pregnancy following assisted reproductive technology (ART) in ATAb-positive euthyroid women. Aspirin and prednisone were used frequently in clinical practice, but the use of these medicines before pregnancy during ART process is still controversial, and the risks of these medicines were not well understood.
Study design, size, duration: A prospective study involving 268 women with unexplained reason for infertility who tested positive for antithyroperoxidase antibody (TPOAb) and/or thyroglobulin antibody (TgAb) were being treated for infertility at the Second Affiliated Hospital of Zhejiang University School of Medicine, Ningbo Women and Children's Hospital and People's Hospital of Jinhua from October 2017 to July 2020. Their TSH level ranged from 0.35-4.0mIU/ml and they all underwent fresh embryo transfer.
Participants/materials, setting, methods: Overall, a total of 268 ATAb-positive women were divided 2 groups: group A: no treatment; B: A+P. Both medicines were used in the lowest effective dose. Between the two groups, we measured oocytes retrieved, fertilization rate, high-quality embryo rate, blastulation rate, cleavage rate,implantation rate, likelihood of clinical pregnancy and miscarriage rate. Kruskal-Wallis test was used in nonnormally distributed variables, and the 2 test or Fisher exact test was used to compare categorical variables.
Main results and the role of chance: A total of 268 infertile women with unexplained reason who tested positive for TPOAb and/or TgAb were recruited in our study. According to assignment, they were divided into two groups. All women in different groups had the similar age, BMI, number of miscarriage and duration of infertility. Levels of FSH, AMH, TSH, FT4, FT3, fibrinogen and d-dimer were similar in all groups. The use of A+P reduced cleavage rate (F=23.982, P<0.001) and implantation rate (F=4.388, P=0.036). The fertilization rate (P=0.407), high-quality embryo rate (P=0.208) and blastulation rate (P=0.157) were not influenced by the use of medication. In this study, likelihood of clinical pregnancy (P=0.066) did not change significantly after therapy, and miscarriage rate (P=0.042) increased after medical treatment.
Limitations, reasons for caution: Firstly, Aspirin is just one representation of anticoagulation therapy, so additional consideration of low molecular heparin should also be considered. Secondly, further randomized controlled trials of aspirin and prednisone alone are needed.
Wider implications of the findings: In this study, use of A+P showed no positive effect, and reduced cleavage rate and implantation rate, while increased miscarriage rate. So, the use of medication for interfile women should be cautious.
Trial registration number: n/a

P-758 The efficacy, safety and proven security of microSecure vitrification offers "peace of mind" and reliability during a global pandemic

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Study question: Under deadly pandemic conditions involving the novel SARS-CoV-2 corona virus, could biopsied blastocysts be safely cryopreserved, stored and utilized for subsequent warming cycles?
Summary answer: Blastocysts were securely stored, effectively warmed and safely transferred to yield normal pregnancy outcomes under uncertain laboratory conditions subject to unprecedented policy changes.
What is known already: By April 2020, every IVF lab worldwide was implementing deep cleaning/disinfecting procedures in their laboratory and patient-contact areas, thorough hand-washing policies and mandatory PPE to reduce the chance of contact transmission and spread of the potentially deadly SARS-CoV-2 coronavirus. Furthermore, we know that safeguards like oil overlay culture dishes and pipetting dilution factors provide insurance against possible contamination. However, knowing that the trophectoderm of blastocysts possessed the ACE-2 binding receptor, potential concern existed regarding the continuation of laser zona opening and biopsy procedures that could possibly expose cryopreserved embryos to the coronavirus in liquid nitrogen storage (vapor or liquid).
Study design, size, duration: Between March 8 and December 22, 2020, 508 patients performed FET cycles involving the use of single (n=490) or dual (n=18) euploid microSecure vitrified blastocysts. In this retrospective analysis, we compared clinical pregnancy outcomes to a 5 year dataset (2015-2019) encompassing 2768 single and 272 dual embryo transfer FET cycles. All blastocysts were vitrified using a closed microSecure system and Innovative Cryoenterprise (ICE; NJ, USA) non-DMSO, glycerol-EG solutions. Differences were assessed by Chi-square analysis (p<0.05).
Participants/materials, setting, methods: Deep cleaning was performed with Simple Green Pro3+ Virucide in non-lab areas (e.g., ET rooms, waiting room) and 6% H2O2 & OoSafe solutions to disinfect lab surfaces and equipment. Group embryo cultures were performed in MCO-5M humidified incubators under low oxygen tri-gas conditions with varying CO2 levels (5.3-6.0%; pH=7.3-7.35) using 25µl droplets of LifeGlobal medium+7.5%LGPS+1%sodium hyaluronate, before changing to 10µl droplet/GPS dishes post-biopsy. FET cycles involved 4-step sucrose dilutions and transvaginal ultrasound-guided embryo transfers.
Main results and the role of chance: While ICSI fertilization rates were unchanged in 2020 (79.4% 2PN vs 77.3%), blastocyst utilization rates tended to be slightly lower than past years (56.4% vs 59.9%) but within an acceptable range. Of 529 blastocysts warmed, 527 (99.7%) survived completely for transfer, being comparable to the 99.4% experienced over 5 years. Furthermore, there was no differences detected in single embryo transfer pregnancy outcomes. The implantation and ongoing clinical pregnancy/live birth rates were 69% and 66.53% compared to 70.4% and 65.1%, respectively. Under pandemic conditions we did not observe an increase in biochemical pregnancies (10.3%) nor spontaneous miscarriage rates (7.8%). Although it is possible that our rigorous disinfection practices could have attributed to lower blastocyst production, the viability of those embryos was not compromised. Importantly, we were able to feel comfortable performing micromanipulation and cryopreservation procedures throughout the year knowing that we were effectively eliminating possible vertical transmission of coronavirus to an exposed trophectoderm layer in cryostorage by applying mircoSecure vitrification. Post-FET clinical check-ups revealed no patient reporting any fever or other Covid-19 symptoms in the weeks following their transfers. We are fortunate to say that our Lab staff, physicians and patients have remained healthy throughout 2020.
Limitations, reasons for caution: Blastocyst survival and viability are independent of possible viral exposure. Previously, the risk of disease transmission via liquid nitrogen or vapor exposure was considered highly unlikely (Pomeroy et al., 2010), but that was at a time when embryos were primarily zona-enclosed. Today's ART standards have us re-evaluating safer approaches.
Wider implications of the findings: We have effectively mitigated avoiding performing zona opening procedures by employing our standard practice of aseptic, closed vitrification. In combination with standard preventative measures (PPE, hand hygiene, distance awareness) and routine deep cleaning practices, we sustained a contamination-free environment and healthy patients, capable of sustaining high levels of pregnancy success.
Trial registration number: Not Applicable

P-759 The impact of the COVID-19 pandemic on women seeking fertility treatment in Germany: the patient´s perspective

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Study question: This cross-sectional survey sought the views of women seeking fertility treatment on the impact of the COVID-19 pandemic on their fertility treatment and attitudes.
Summary answer: Most respondents worried the recommendations to postpone treatment would reduce their chances of pregnancy and were concerned about negative effects of SARS-Cov-2 infections on pregnancy.
What is known already: In spring 2020, the recommendations of ESHRE and German professional societies to postpone fertility treatments resulted in limited or closed services from mid-March to early May in many clinics. Previous studies reported that postponing fertility clinic appointments causes psychological distress, with most patients saying they would have preferred to continue treatment. While no similar studies are available from Germany, where COVID-19 incidence was relatively low, concerns of the patients about possible consequences of a treatment delay and a SARS-CoV-2 infection on fertility and pregnancy remain unknown.
Study design, size, duration: This cross-sectional, anonymous, online questionnaire was completed in June-December 2020 by 249 women attending fertility clinics across Germany. The women were recruited using leaflets, directly by study personnel either in person or by telephone, or via online support group forums for fertility patients.
Participants/materials, setting, methods: All women seeking treatment in fertility clinics were eligible to participate. The online survey covered questions about the patient's quality of life, their opinions about the professional societies' recommendations and their effects as well as any concerns about infection with SARS-CoV-2. Statistical analysis was conducted using SPSS Version 27.
Main results and the role of chance: Three-quarters of participants disagreed with the pausing of fertility treatments. Women who participated from October-December 2020, when the incidence rate was high, were as likely to disagree as participants that participated from June-September 2020 (73% vs 79%, p=0.3). Nearly all participants (95%) were concerned that treatment delays would reduce their chances of pregnancy. 72 participants (29%) had their appointments cancelled. Nearly all (97%) reported being upset by this, with 40 (56%) reporting that they were extremely or very disappointed about the cancellation. Women coming for follow-up appointments and women who had to wait 10 weeks or longer were more likely to be upset by the postponement or cancellation of their appointment (p=0.016 and p=0.012, respectively).

Nearly all (97%) of the participants were worried about possible negative effects a SARS-CoV-2 infection might have related to their fertility, pregnancy or unborn child. Sixty-one percent stated they were very or moderately concerned about the negative influence the infection might have on the woman´s own health during pregnancy and 60% were very to moderately concerned about potential negative effects for the unborn child. However, only 26% reported they were very or moderately concerned about the potential negative effects of an infection on fertility.
Limitations, reasons for caution: The main limitations of this study are the possibility of selection bias as people with strong opinions are more likely to have participated and the lack of information on non-responders due to the study design. Also, the Covid-19 pandemic is evolving continuously meaning that participants´ opinions may vary over time.
Wider implications of the findings: Postponement of treatments increased distress among patients and should be avoided when possible. If unavoidable, follow-up patients should be prioritised for treatment and the length of postponement should be minimised. Fertility clinics must provide information about the current state of knowledge of SARS-Cov-2 infections in pregnancies and options for immunization.
Trial registration number: not applicable

P-760 Cycle outcomes in frozen-thawed single blastocyst transfers in overweight and obese women

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Study question: Are cycle outcomes different in frozen embryo transfer in obese and overweight women compared to normal weight cases?
Summary answer: As BMI increases, although implantation rates are similar, miscarriage rates increase and live birth rates decrease, especially in cases whose BMI value is above 30.
What is known already: Obesity has adverse effects on the reproductive system. Obesity causes ovulatory dysfunction and menstrual cycle disorders, thus reducing fertility. As BMI increases, the implantation, pregnancy and ongoing pregnancy rates decrease and the rate of clinical losses increases. In donor oocyte cycles, the BMI of the recipient has a statistically significant detrimental effect on obstetrics outcomes such as pregnancy rate and live birth rate. However, very few studies evaluate the impact of BMI on frozen-thawed single blastocyst transfer.
Study design, size, duration: This retrospective study was conducted at Istanbul Memorial Hospital, ART and Reproductive Center between 2011 and 2020. A total of 5642 frozen-thawed single blastocyst transfer cycles were examined. Patients were grouped according to the World Health Organisation BMI classification system: Group I (BMI 25-29.9) (n=1663); Group II (BMI 30-34.9) (n=598); Group III (BMI 35-39.9) (n=150); Group IV (BMI>40) (n=30); Control Group (BMI 18.5-24.9) (n=3201). Participants/materials, setting, methods: Patients between 17-42 years old were included. Preimplantation genetic diagnosis (PGD) was performed for patients >37 years old. Exclusion criteria were: repeated pregnancy losses, Mullerian abnormalities, intrauterine adhesions, endometrial thickness <7mm during frozen embryo transfer (FET) cycle. For endometrial preparation, modified natural cycle or artificial cycle were used. Single top/good quality blastocysts were transferred.
Main results and the role of chance: A total of 5642 FET cycles were analyzed. There was no significant difference in patient characteristics in terms of mean age, endometrial thickness on embryo transfer day and Anti Mullerian Hormone levels between the groups. Cycle outcomes were analysed according to the BMI groups. Mean age of groups were 32.1(17-42), 32(18-42), 32.6(20-42), 32.8(23-42) in groups I to IV respectively and 32(18-42) in the control group**.**

Pregnancy rates were 70.9%(n=1180) 70.7%(n=423), 76%(n=114) and 54.8%(n=17) in groups I to IV respectively and 72.4% in the control group(n=2321) (p>0,05). **Clinical pregnancy losses rates** were 18.9%(n=196), 23.9%(n=86), 23.1%(n=22) and 23%(n=3) in groups I to IV respectively and 15.1% in the control group(n=316) (p<0,05). The **live birth rates** were 49.9%(n=830) 45.1%(n=270), 47.3%(n=71) and 33.3%(n=10) in groups I to IV, respectively and 54.9% (n=1759) in the control group (p<0,05).

There was no statistically significant difference in implantation rates between the groups but clinical pregnancy losses rates were higher in obese patients (groups II-III-IV) whereas live birth rates were lower compared to group I (overweight) and the control group.
Limitations, reasons for caution: The limitation of the study is its retrospective nature.
Wider implications of the findings: Our study shows that, there is a significantly higher risk of negative cycle outcomes in obese patients. Pre-treatment counselling is therefore needed to increase patient awareness of the risks and to provide advice on weight loss.
Trial registration number: not applicable

P-761 Can modified luteal support in fresh cycle after agonist trigger "rescue" the cumulative live birth rate (CLBR) in high responders

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Study question: Can modified luteal support in fresh cycle "rescue" the cumulative live birth rate (CLBR) in high responders who receive agonist trigger?
Summary answer: Live birth rate in high responders who had agonist trigger in fresh cycle was significantly reduced despite modified luteal support.
What is known already: Previous studies, including small randomised controlled trials, claimed that good live birth rate could be achieved at fresh transfer in "high responders" who had GnRHa trigger with modified luteal phase support. However, majority of these studies exclude the true high responders (patients with 20 and above oocytes) and average number of collected eggs reported in many of these studies in the range of 9 to 12. The data on outcome of fresh transfer in true high responders is very limited.
Study design, size, duration: A prospective observational study was conducted in 407 patients, aged 23–42 years who were expected to be at risk of high response (AFC>18, AMH>20 pmol/l) undergoing controlled ovarian stimulation between 2014-2019 triggered either with HCG or GnRH agonist. Live

birth rate (LBR) in a fresh and subsequent 3 frozen transfers were compared in groups with different triggers and freeze all.
Participants/materials, setting, methods: Patients were stimulated in short antagonist protocol. The trigger was chosen based on the background characteristics, peak oestradiol and clinician discretion. Triggering was achieved either with 0.5 mg buserelin (GnRHa) 0.5mgin 230 patients (A) or with 250 mcg of hCG(H) in 177 patients. Modified luteal support included vaginal progesterone, oral oestrogen and 1500 iu of hCG on the day of egg retrieval. The later was omitted with more than 20 oocytes.
Main results and the role of chance: The mean age, AFC, number of previous cycles, number of embryos transferred were 33.3, 22.4, 0.26 and 1.2 respectively and did not have significant difference between different triggers. Whereas AMH (53 pmol/l (A) vs 43.1 pmol/l (H), P=0.003), peak oestradiol (15140.74 (A) vs 9738.59 (H), P=5.59X10-14), and number of oocytes collected (21 vs.17, P=5.63X10-7) were significantly higher in GnRHa group. Seventeen patients in buserelin group had elective freeze all. Ovarian Hyperstimulation Syndrome (OHSS) rate was 3.9% in buserelin group (more then half of these cases had a bolus of hCG at egg collection; most were mild/moderate). On the other hand, hCG group had 2.5% of OHSS (all severe). Live birth rate in fresh cycle was 31% in hCG and 21% in GnRHa groups. If freeze all was undertaken in fresh cycle after GnRHa trigger, then LBR in the first frozen cycle of this group was 53% (P=0.003, fresh vs frozen GnRHa group). CLBR was not different between GnRHa and hCG groups (51%). However, this was significantly lower than CLBR in GnRHa trigger freeze all group 76% (P=0.03)
Limitations, reasons for caution: The limitation of this study is its non-randomised nature. However, since it is one of the biggest studies for high responders it has a power to minimise bias by adjusting for multiple variables.
Wider implications of the findings: Proceeding with fresh transfer in high responders after GnRHa trigger with modified luteal support not only maintains the risk of OHSS (equivalent to hCG group) but also significantly impairs the LBR not compensated even after 3 subsequent frozen embryo transfers. Therefore, freeze-all approach should be preferred in this group.
Trial registration number: NA

P-762 Preeclampsia in pregnancies resulting from oocyte donation, IVF or natural conception. A systematic review and meta-analysis

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Study question: What is the prevalence of preeclampsia (PE) in pregnancies after oocyte donation (OD) compared to natural conception (NC) and to IVF with autologous oocytes?
Summary answer: PE prevalence in singleton pregnancies after OD was five times higher than in NC and almost three times higher than after IVF with autologous oocytes. What is known already: The indication for OD is expanding to lesbian women requesting shared lesbian motherhood. Previous reviews showed that the risk of PE is higher in pregnancies after OD than after IVF with autologous oocytes and natural conception. Classification on severity of PE is lacking as is the relationship with known risk factors such as maternal age and multiple gestations. Furthermore the actual prevalence of PE following in pregnancies resulting from OD is not known.
Study design, size, duration: Systematic review and meta-analysis. A literature search was performed using the following databases: PubMed, EMBASE and CINAHL, OpenGrey and Greynet from January 1980 through July 2020.
Participants/materials, setting, methods: We included retrospective and prospective cohort studies. The study population consisted of pregnancies after OD and NC or IVF and data had to be available about prevalence of PE. We compared the risk of (severe) PE in OD versus NC and IVF pregnancies, subgrouped by parity and maternal age. We calculated individual and pooled odds ratios (OR) and prevalence estimates with 95% CI using a random effect model, while heterogeneity was assessed by the I2.
Main results and the role of chance: We included 28 studies comprising of 7131 OD pregnancies, 1.139.540 NC pregnancies and 72.763 IVF pregnancies were available for analysis. The risk of PE and severe PE was increased in OD pregnancies compared to NC pregnancies (pooled OR of all subgroups: 5.09, 95% CI: 4.29 – 6.04; I2 = 19% and OR: 7.42 (95% CI: 4.64-11.88; I2 = 49%). The pooled adjusted OR for PE was 3.24 (95% 2.74 – 3.83) for OD versus natural pregnancies.The risk of PE and severe PE was increased in OD pregnancies compared to IVF pregnancies (pooled OR of all subgroups: 2.96, 95% CI: 2.49 – 3.51; I2 = 51% and OR: 2.97, 95% CI: 2.15 – 4.11; I2 = 0%). The pooled adjusted OR for PE was 2.67 (95% 2.28 – 3.13) for OD versus IVF.The pooled prevalence of PE in singleton pregnancies after OD was 10.7% (95% CI 6.6 – 15.5) compared to 4.1% (95% CI 2.7 – 5.6) after IVF and 2% (95% CI 1.0 – 3.1) after NC. The prevalence in multiple pregnancies was 27.8% (95% CI 23.6 -32.2) after OD, 9.7% (95% CI 6.2 – 13.9) after IVF and 7.5% (95% CI 7.2 – 7.8) after NC.
Limitations, reasons for caution: The precise definition of PE is still a matter of debate. The different criteria could have affected the prevalence estimate.
Wider implications of the findings: Nearly one in six women will suffer PE after OD. Women who can conceive naturally i.e. shared lesbian motherhood, should be discouraged to turn to OD. In women with premature ovarian failure factors that increase risk of PE should be avoided. We suggest therefore single embryo transfer.
Trial registration number: not applicable

P-763 Neonatal outcomes of the first 65 infants delivered after IVF treatment with progestin-primed ovarian stimulation using dienogest in patients with endometriosis

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Study question: What is the perinatal outcome of pregnancies resulting from a controlled ovarian hyperstimulation (COH) regimen of progestin-primed ovarian stimulation (PPOS) protocol using dienogest (DNG) in patients with endometriosis?
Summary answer: No difference in mean birth weight, however preterm and low birth weight babies are significantly more in the group treated with PPOS using DNG.
What is known already: Dienogest is an oral progestin effective for the treatment of endometriosis, such as reduction of endometrial lesion and control of pain intensity with safety profile and good tolerability. We reported for the first time in the world that DNG was better than dydrogesterone (DYG) for PPOS in terms of the mature oocytes rate and the fertilization rate in patients with endometriosis. Although there have been several reports of infants born with PPOS using DYG, it is essential to report on the perinatal outcome of embryos transferred after treatment with PPOS using DNG from now on. Study design, size, duration: We prospectively investigated the perinatal outcomes of 65 newborns which were the result of using a new COH regimen; PPOS with DNG. The results were compared with perinatal outcome data of babies born between 2018 and 2020 to 815 patients who underwent assisted reproductive technology (ART) treatment at our fertility center. As for the congenital malformation rate, the data was also compared with the 2017 Japanese data bank of babies born after ART treatment.
Participants/materials, setting, methods: We studied the perinatal data of all babies born after transfer of frozen embryos acquired by COH using PPOS protocol with DNG. The rate of maternal complications during pregnancy, pregnancy duration, birth weight, congenital malformations and method of delivery were investigated. We compared the perinatal outcomes of infants born after *in vitro* fertilization (IVF) and frozen embryo transfer at our center during the same period.
Main results and the role of chance: Perinatal data of 65 babies (study group) were compared with the perinatal data of 840 babies born after IVF at our center, and 47807 babies born after ART in Japan, 2017. We found 3 twin and 59 singleton pregnancies in the study group, compared to 23 twins, 1 triplet and 791 singleton pregnancies during the same period at our center. Considering singletons, there was no difference in mean birthweight (study group; 2893.2±652g vs. total at our center; 3001.2±425g, respectively, p=0.102). Preterm births (<37 weeks) were significantly more frequent in the PPOS using DNG treatment group than in total at our center (19.2% vs. 9.7%, p=0.016). The percentage of infants with a birth weight < 2.5 kg was also significantly higher in the PPOS treatment group compared to the total at our center (22.6% vs.11.9%, p= 0.015). The Caesarean section rate was 53.2% in the study group vs. 47.1% control group of our center respectively (p=0.353). One babies in

the study group had malformations in the ocular region. There was no significant difference in congenital malformations between the study group and ART data bank in Japan, 2017 (OR 0.67, 95% CI 0.093: 4.836).
Limitations, reasons for caution: The number of babies is still low, further prospective studies including larger populations are needed to confirm the efficacy of PPOS protocol with DNG.
Wider implications of the findings: This is the first report on the perinatal outcome of babies born by a new COH method using PPOS with DNG, which is a combination of endometriosis treatment and COH for IVF. The association of endometriosis with preterm birth and low birth weight needs to be further investigated.
Trial registration number: UMIN000031111

P-764 Risks of oocyte donation for third-party donors: a systematic literature review

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Study question: What are the risks of oocyte donation? Which risks should be prioritised in policies aiming to improve the protection of third-party oocyte donors?
Summary answer: The risks for third-party oocyte donors are of a diverse nature, including physical risks, psychological risks, iatrogenic risks, and social risks.
What is known already: Oocyte donation involves ovarian stimulation and oocyte pick-up, which represent burdensome procedures for the donor. In a recent evaluation of the EU legislation on blood, tissue and cells, the European Commission highlighted that oocyte donors are currently not adequately protected. For effective oocyte donor protection measures to be developed and implemented, it is important to understand the risks that oocyte donors are exposed to. To date, there is no comprehensive overview of the existing knowledge on the physical and psychosocial risks of oocyte donation.
Study design, size, duration: A systematic literature review of the publications on PubMed, CINAHL, PsycINFO and the Notify Library was carried out. The search was conducted in May 2020. All empirical studies, including case reports, that reported or investigated negative experiences of oocyte donors and/or negative consequences of the donation on the donors' physical health, mental health, or other aspects of their lives were included. No restriction was made with regard to the year of publication.
Participants/materials, setting, methods: In total, 88 empirical studies conducted in oocyte donors were reviewed. All reported information on oocyte donor risks was extracted and summarised. The identified risks were clustered into categories according to common themes and analysed with regard to their frequency of occurrence, severity, and imputability to the donation. A prioritisation of risks was carried out based on these three criteria, classifying each risk as a "priority risk" or a "non-priority risk".
Main results and the role of chance: Nineteen priority risks were identified across the following six categories: short-term physical risks, long-term physical risks, short-term psychological risks, long-term psychological risks, iatrogenic risks, and social risks. The most frequently reported priority risks were moderate to severe Ovarian Hyperstimulation Syndrome (OHSS) and having lasting worries or concerns about the donation. While the findings confirmed the relevance of certain immediate physical risks for oocyte donors, no cases of death or permanent physical damage as a direct consequence of the donation could be detected. The results showed that donating oocytes can profoundly impact the donors' psychological well-being in the short-term and in the long-term. Furthermore, the donation can have a strong effect on the donor's social and family life, for instance, through the risk of unintended pregnancy. Moreover, it was found that oocyte donors are at risk of experiencing mistreatment or inadequate care during the donation procedure. Most studies included in the review reported on short-term risks of the donation. There is a high degree of uncertainty about the long-term health effects of oocyte donation. Due to the scarcity of large observational studies, the conclusions are mostly based on small studies and case reports, which limits the strength of any conclusion.
Limitations, reasons for caution: The literature search was limited to common databases for published data. Grey literature was not searched. Due to the heterogeneous nature of the relevant publications, it is possible that the search strategy was not able to detect all eligible articles.
Wider implications of the findings: The findings emphasise the importance of implementing effective donor protection policies that address not only the physical, but also the psychological, social, and iatrogenic risks of oocyte donation. Moreover, the findings call for a systematic follow-up of oocyte donors to gain insight into the long-term consequences of the donation.
Trial registration number: Not applicable

P-765 Embryo donation pregnancies are at high risk even in young recipients

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Study question: Are pregnancies after embryo donation (ED) at higher risk of complications than those issued from autologous frozen-thawed embryo transfer (FET)?
Summary answer: Even in young women, the risk of pregnancy induced hypertension (PIH) is four time higher in pregnancies after ED versus controls.
What is known already: After oocyte donation, a higher risk of PIH is well described. It is more controversial after sperm donation. The risk origin remains uncertain, even though an immunological explanation seems most likely. In ED, the fetus being fully allogeneic to his parents may be less well-tolerated. Very few data are reported about pregnancies after ED. The same allogenic model exists in surrogacies, but pregnancy complications are not well described in the literature.
Study design, size, duration: This anonymous, multicenter, comparative observational retrospective cohort study included all singleton ED pregnancies diagnosed at 7–8 weeks, from January 2003 to December 2018, in six French ART centers. For each, two controls were matched among autologous FET pregnancies. 209 pregnancies were included: 73 ED and 136 controls. Multiple pregnancies were excluded because of their increased associated obstetrical risks.
Participants/materials, setting, methods: Controls were matched according to pregnancy date, parity and women's age. The first two singleton pregnancies after each index case meeting the selection criteria were retained. Each center coordinator collected information on infertility, pregnancy pathologies, outcomes and newborns. Statistical methods included univariate and multivariate analyses. According to French practice, all women were under 44 y/o. The main outcome was the percentage of PIH for ED versus controls.
Main results and the role of chance: ED was indicated for genetic disease in 17 cases (23.3%), double total infertility in 28 cases (38.3%), and double partial/total infertility in 35 cases (47.9%). Groups were comparable in age (mean age: 34.5 ± 8.6 versus 34.5 ± 4.5; p=0.68), BMI, except for parity (more nulliparity in ED group: 90.4% vs 79.4%; p=0.04). Pregnancy outcomes were similar for ED and control groups, the percentages of deliveries being 80.8% and 83.8%, respectively (p=0.58). PIH occurred significantly more frequently among ED than control pregnancies (24.6% versus 11.9%; *P*= 0.04), with the difference mainly observed for severe forms: preeclampsia and HELLP (17.5% vs 4.6%; p=0.01). No eclampsia was reported. In contrast, isolated hypertension frequency was comparable (7.0% vs. 7.3%, p=0.94). Regarding labor and delivery mode, in ED group C-section was more frequent (47.3% vs 29.2%; p= 0.03). In

neonatal data, no difference was found between ED and control group for prematurity, weight and height at birth, Apgar score, Small for gestational age, Large for gestational age and sex ratio. Seven neonatal malformations were recorded in ED group and 3 in the control group (NS).

Limitations, reasons for caution: Retrospective study in a relatively long period when different endometrial preparation for frozen-thawed embryo transfer and embryo cryopreservation method were used. Relatively limited number of ED because of low practice in France. No analysis of embryo stage at transfer (cleaved embryo or blastocyst).

Wider implications of the findings: The PIH risk must be acknowledged to inform couples and provide careful pregnancy monitoring. A special care for gestational carrier should also be done since the allogenic situation is the same than in ED recipients.

Trial registration number: not applicable

P-766 Neurodevelopment in fetuses conceived by assisted reproductive technologies following fresh and frozen embryo transfer

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Study question: Do *in vitro* fertilization (IVF) offspring present different neurodevelopment assessed by fetal neurosonography and infant neurobehavioral tests as compared to those spontaneously conceived (SC)?

Summary answer: IVF offspring, especially those obtained after fresh embryo-transfer (ET), showed subtle structural differences in fetal neurosonography and poorer neurobehavioral scores at twelve months of age.

What is known already: The number of pregnancies following assisted reproductive technologies (ART) is currently increasing worldwide. Concerns about the neurodevelopment of subjects conceived by IVF have been rising and mostly studied in children and adolescents with inconsistent results. Many of the identified risk associations were only observed in subgroups or disappeared after adjustment for covariates, mainly multiple pregnancy and gestational age at birth. It is unknown whether fetal brain development and cortical folding differ prenatally in IVF fetuses as compared to SC.

Study design, size, duration: This is the first study examining fetal neurodevelopment by neurosonography in IVF fetuses. A prospective cohort study of 210 singleton pregnancies recruited from 2017 to 2020, including 70 SC gestations, 70 conceived by IVF following frozen ET (FET) and 70 IVF after fresh ET. Fetal neurosonography was performed in all pregnancies. Additionally, *Ages & Stages Questionnaires* (ASQ) were obtained at 12 months of corrected age.

Participants/materials, setting, methods: IVF pregnancies were recruited from a single Assisted Reproduction Center, ensuring homogeneity in IVF stimulation protocols, endometrial preparation, laboratory procedures and embryo culture conditions. SC pregnancies were randomly selected from low-risk fertile couples and paired to IVF by maternal age. Fetal neurosonography including transvaginal approach was performed at 32±2 weeks of gestation, measured offline by a single investigator and normalized by biparietal or occipitofrontal diameter. ASQ were obtained postnatally, at 12 months of corrected age.

Main results and the role of chance: Study groups were similar and comparable regarding maternal age, body mass index, study level and employment rate together with exposure to smoke, alcohol, aspirin and corticoids during pregnancy, gestational age (32±2 weeks) and estimated fetal weight (1700±400g) at neurosonography.

As compared to SC pregnancies, both IVF populations showed differences in cortical development with reduced parieto-occipital (fresh ET 12.5mm [SD 2.5] vs FET 13.4 [2.6] vs SC 13.4 [2.6]), cingulate (fresh ET 5.8 [IQR 4.2-7.4] vs FET 5.8 [4.1-7.5] vs SC 6.5 [4.8-7.8]) and calcarine (fresh ET 13.5 [IQR 10.1-16.1] vs FET 14.5 [12.1-15.8] vs SC 16.4 [14.3-17.9]) sulci depth together with lower Sylvian fissure grading. Cortical development changes were more pronounced in the fresh ET group as compared to FET. Corpus callosum length and insula depth were lower in FET and fresh ET groups, respectively. Neurosonographic changes remained statistically significant after adjustment by ethnicity, gender, gestational age and weight centile at scan.

IVF infants showed worse ASQ scores, especially in fresh ET for communication, personal-social, fine-motor and problem-solving skills. Gross-motor scores were significantly lower in FET as compared to SC and fresh ET. Differences were statistically significant after adjustment by maternal ethnicity, study level, employment status, breastfeeding, gender and corrected age.

Limitations, reasons for caution: The reported neurodevelopmental differences are subtle, with most neurosonographic findings lying within normal ranges. Infertility factors contribution to the outcome cannot be unraveled from the ART procedure itself. The milder features found in FET individuals cannot condition the technique´s choice and must be considered together with their global perinatal results.

Wider implications of the findings: Neurosonography is an appropriate tool to identify subtle brain differences between fetuses exposed and not exposed to ART. Prenatal features were consistent with postnatal neurobehavioral findings. These results support the relevance of a neurodevelopmental follow-up in IVF patients. Further studies are warranted to assess the long-term performance in these subjects.

Trial registration number: not applicable

P-767 Cumulative live birth rate after IVF - trend over time and the impact of blastocyst culture and vitrification

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Study question: Has cumulative live birth rate (CLBR) improved over time and which factors are associated with such an improvement?

Summary answer: During 2007-2017, CLBR per oocyte aspiration increased significantly (27.0 % to 36.3 %), in parallel with an increase in blastocyst transfer and cryopreservation by vitrification.

What is known already: While it has been shown that live birth rate (LBR) per embryo transfer (ET) is higher for fresh blastocyst than for fresh cleavage stage embryo transfer, CLBR per oocyte aspiration, including one fresh ET and all subsequent frozen embryo transfers (FET), does not seem to differ between the two culture strategies.

Study design, size, duration: STUDY DESIGN, SIZE, DURATION: National register study including all oocyte aspirations performed in Sweden 2007-2017, n=124 700. Donation cycles excluded.

Participants/materials, setting, methods: Data were retrieved from the Swedish National Registry of Assisted Reproduction (Q-IVF). CLBR was defined as the number of deliveries with at least one live birth resulting from one oocyte aspiration, including all fresh and/or frozen embryo transfers within one year. The delivery of a singleton, twin, or other multiples was registered as one delivery. Cryopreservation of cleavage stage embryos was performed by slow freezing and of blastocyst by vitrification.

Main results and the role of chance: Overall, the CLBR per oocyte aspiration increased significantly during the study period, from 27.0 % to 36.3 % (OR 1.039, 95% CI 1.035-1.043) and from 30.0 % to 43.3 % if at least one ET was performed (AOR 1.055, 95% CI 1.050-1.059). The increase in CLBR was independent of maternal age, number of oocytes retrieved and number of previous IVF live births. The CLBR for women <35 years and ≥ 35 years both increased significantly, following the same pattern. During the study period a substantially increasing number of blastocyst transfers were performed, both in fresh and in FET cycles. An important contributor included in the blastocyst strategy, may be the extended culture of the total cohort of embryos, also embryos earlier discarded at early cleavage stages, in order to reach the blastocyst stage. These embryos may contribute to the total number of available blastocysts and thereby increase the chance of a live birth within that oocyte aspiration cycle. Other important predicting factors for live birth, such as number of embryos

transferred, could not explain the improvement, on the contrary the single embryo transfer (SET) rate increased with time.
Limitations, reasons for caution: The retrospective design implicates that other confounders of importance for CLBR can not be ruled out. In addition, some FET cycles might be performed later than one year post oocyte aspiration for the last year (2017) and are thus not included in this study.
Wider implications of the findings: The results suggest that blastocyst transfer, particularly when used in FET cycles and in combination with vitrification, is an important contributor to the improved live birth rates over time. This gives a possibility for fewer oocyte aspirations needed to achieve a live birth and a shortened time to live birth.
Trial registration number: -

P-768 Childhood growth of singletons conceived following in vitro fertilization (IVF) - does gonadotropin-stimulation matter?

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Study question: Is there a difference in growth or weight gain of children conceived after IVF with or without gonadotropin-stimulation compared to standard growth references? Summary answer: We observed no difference in growth between children conceived after IVF with or without gonadotropin-stimulation and spontaneously conceived children.
What is known already: In recent studies, singletons conceived after IVF cycles had lower birth weight than spontaneously conceived singletons. The etiology of the impaired intrauterine growth is unclear, but insufficiency of placental function or possible epigenetic effects is discussed. Data regarding normalization or continuation of reduced birth weight are controversial. The growth of children born after unstimulated natural cycle IVF (NC-IVF) has never been studied.
Study design, size, duration: Single-center, university based cohort study. 139 singletons born after NC- IVF and children born after conventional gonadotropin stimulated IVF (cIVF) in 2010 -2017 were studied. Stimulation dosage in cIVF was ≥150 IU/d human gonadotropin.
Participants/materials, setting, methods: We collected weight, length and head circumference at birth and at one, two, four, six, 12, 18 and 24 months. We calculated standard deviation scores based on national growth references. Growth parameters (weight, length and head circumference) were compared between NC-IVF and cIVF singletons (stimulated with ≥150 IU/d human gonadotropin) using Mann-Whitney U tests.
Main results and the role of chance: In general, growth of children conceived after IVF did not differ from national references. Of the 139 singletons conceived, 98 singletons were conceived after NC-IVF and 41 after cIVF. The parents did not differ in ethnicity, age, BMI or health status between groups, and there was no significant difference in gestational age, pregnancy complications and smoking or breastfeeding habits either. The median birth weight in NC-IVF children was 3.4kg (0.1 standard deviation score, SDS) and in cIVF 3.3kg (-0.3 SDS) (p=0.53). Median length at birth in NC-IVF was 50cm (-0.5 SDS) and did not differ from cIVF children 50cm (-0.8 SDS) (p=0.52). At age 12 months, the median weight was 9.3kg (0.0 SDS) for NC-IVF children compared to 9.0kg (-1.7 SDS) for cIVF children (p=0.44). Median lengths was 75cm (0.1 SDS) in NC-IVF versus 71cm (-1.6 SDS) in cIVF children (p=0.89). At age 24 months, median weight in NC-IVF children was 12.3 kg (0.3 SDS) versus 10.5 kg (-1.2 SDS) in cIVF (p=0.72) and median lengths 87.5cm (0.1 SDS) in NC-IVF versus 87.6 cm (0.1 SDS) in cIVF children. These discrete non-significant differences in weight and length gain compared to standardized growth curves and between the two groups are reassuring.
Limitations, reasons for caution: Willingness to participate is prone to selection bias. Further studies with larger samples are needed to confirm these findings.
Wider implications of the findings: This is the first study investigating weight and length gain in children after unstimulated IVF. Growth is an important proxy for the health of children. These reassuring results are of imminent importance for the children born after IVF and their parents.
Trial registration number: BASEC (ID 2015-00235)

P-769 FTIR spectroscopy analysis reveals differences between human embryo culture media composition by type of formulation and by manufacturer

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Study question: Can we detect the variation in different commercial human embryo culture media composition by Fourier-transform infrared (FTIR) spectroscopic analysis?
Summary answer: The spectra reveals distint features that allow distinguishing between continuous and sequential media, as well as between manufacturers of the same media type. What is known already: Manufacturers do not fully disclose commercially available culture media formulations. For this reason, it is important to gain insight into de differences between the available formulations, in order to understand how they might be linked with efficacy and safety ART parameters. Fourier Transform Infra-Red (FTIR) can be used for this purpose.
Study design, size, duration: Culture media samples (1 mL) were collected from local IVF laboratories, and stored frozen at -20 °C. Five repeats of 25 µL aliquots from the samples were analysed using FTIR spectroscopy to acquire the whole molecular fingerprint of each culture media.
Participants/materials, setting, methods: Three continuous (SAGE 1-STEP, Origio, G-TL, Vitrolife, and GERI, Genea) and four sequential (G1 PLUS, Vitrolife; Sequential Cleav with phenolred, Origio; G2 PLUS, Vitrolife and Sequential Blast with phenolred, Origio) media were analysed. Different pre-processing methods (atmospheric and baseline correction, normalizations, and derivatives) were carried out to minimize physical artefacts while highlighting chemical features. To compare the spectra of different media, multivariate analysis, as principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed.
Main results and the role of chance: The whole molecular fingerprint of all media analysed showed a similar pattern, revealing that, overall, the composition is very similar. However, PCA and HCA analysis revealed that significant differences exist, both between media type (continuous vs. sequential), and between different manufacturers within the same media type. Average linkage clustering using Spearman's rank correlation confirms the similarities between the continuous and the sequential formulations. An analysis focusing the fingerprint region of the spectra (900 – 1700 cm^-1), also revealed variability between manufacturer, between media type (continuous vs. sequential) and of stage-specific media (cleavage vs. blastocyst). For instance, GERI media visually appeared to have distinct peaks compared to all other media, which was confirmed later through multivariate statistical analysis.
Limitations, reasons for caution: FTIR spectroscopy does not allow for a direct identification of the analytes present in the culture media, we can only infer the functional groups, but that are common on diverse biomolecules.
Wider implications of the findings: FTIR analysis reveals differences between different media, such as cleavage and blastocyst specific, sequential and continuous, or manufacturer's formulations. The increased resolution of the FTIR profile proves to be a powerful tool for analysing human embryo media, and could be used to establish correlations with media clinical performance and safety.
Trial registration number: not applicable

P-770 Two in One - Monozygotic splitting and associated perinatal outcomes after oocyte freezing; an exploratory analysis of the UK national database from 1990 to 2016

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Study question: Is oocyte freezing a risk factor for monozygotic splitting?
Summary answer: There is a trend towards a higher monozygotic splitting incidence among frozen oocytes, but this did not reach statistical significance.
What is known already: Laboratory techniques which involve embryo manipulation such as ICSI, assisted hatching, embryo biopsy for pre-implantation genetic testing and extended culture to the blastocyst stage appear to increase the risk of monozygotic splitting. Whilst there is some data that embryo freezing does not appear to increase the risk of monozygotic splitting, there is no comparable analysis on whether oocyte freezing increases the risk of monozygotic splitting.
Study design, size, duration: This was a retrospective cohort study analysing 988 015 ART (assisted reproductive technique) cycles from the HFEA anonymised database from 1990 to 2016. As frozen oocytes require ICSI, only fresh oocytes with ICSI were taken for comparison and frozen embryo transfers were excluded. Only single embryo transfers were included.[CM1] [MM2] We also noted ages of the female partner at the time of treatment, stage of embryo transfer, and whether pre-implantation genetic testing had been performed.
Participants/materials, setting, methods: There were 84 085 ICSI cycles with single embryo transfers using fresh oocytes and 596 using frozen oocytes. Monozygotic splitting was defined as the presence of two foetal hearts [CM1] [MM2] on ultrasound. Live birth (LB)was defined as either a singleton or a twin LB resulting from a monozygotically split embryo. Preterm birth (PTB) was defined as birth prior to 37 weeks gestation and early PTB as birth prior to 32 weeks gestation.
Main results and the role of chance: The frozen oocyte group had fewer women in the under-35 age group (frozen oocytes 16.6% vs fresh oocytes 53.6%, p<0.0001) and a higher proportion of blastocyst transfers (frozen oocytes 55.1% vs fresh oocytes 48.8%, p=0.002) There were only 10 PGT cycles amongst monozygotically split embryos from fresh oocytes in our analysis, and none in the frozen oocyte group. Hence, this was not included as a confounder. There was a non-significant trend toward a higher incidence of monozygotic splitting amongst frozen oocytes (4/596, 2.3%, all monozygotic twins) than amongst fresh oocytes (378/27 019, 1.4%, 372 monozygotic twins and 6 monozygotic triplets); OR 1.688, 95% CI 0.623 to 4.574 and aOR 1.506, 95% CI 0.531 to 4.274 (maternal age and stage of embryo transfer adjusted as confounders). Of the 378 monozygotically split embryos from fresh oocytes, 308 (81.5%) had a LB: of which 47 (15.3%) were singletons and the rest were twins; 241 (78.2%) were PTB and 56 (18.2%) were early PTB. Of the four monozygotic twins from frozen oocytes, all reached a LB; one was a singleton term LB (Birthweight 3-3.5kg) whilst three were twin preterm LBs at 35-36 weeks, with no early PTBs and twin median birthweight 2-2.5 kg.
Limitations, reasons for caution: Albeit a large national database, this cohort study was restricted due to absence of data on potential confounders such as age at oocyte freezing, method of cryopreservation and length of storage. [CM1] Data was also lacking on amnionicity, obstetric risks including pre-eclampsia, twin-to-twin-transfusion syndrome, intrapartum and late effects.
Wider implications of the findings: With rapid rise in egg freezing, our findings would help reassure women that eggs on ice does not predispose to significant risk of two-in-one monozygotic splitting. However, the marginal trend (from 1.4% in fresh to 2.3% in frozen oocytes), does indicate that this subject merits further research.
Trial registration number: Not applicable. A database based retrospective study

P-771 I hear, I forget. I see and I remember. I do and I understand. An Insight into the need for training for add on techniques

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Study question: Do embryologists need additional training or certifications before using add on techniques in the lab ?
Summary answer: Out of 173 respondents majority feel add on techniques require training and/or certifications, the mode of training varies for different add ons.
What is known already: Cochrane reviews have suggested that minimal evidence exists for the use of add on treatments in ART, the data on the prevalence of add ons in IVF is unclear but the presence of technologies in ART laboratories world over suggests a increasing trend of adoption of unproven techniques. No data exists suggesting the role of embryologists in performing the Add on techniques and how their training or lack of, can impact patient safety. The most common method of training comes from the manufacturers and there is a lack of structured trainings for add on treatments worldover.
Study design, size, duration: An internet based survey was designed keeping in mind commonly available laboratory add ons. It comprised of 9 sections and a total of 18 multiple choice questions. Answer choices ranged from a simple yes or no to more complex choices suggesting the type of training and the potential benefits of training.
Participants/materials, setting, methods: The Survey includes results from 173 embryologists from india with varying degree of experience. Add ons included in the survey were Sperm DNA Fragmentation test, IMSI, PICSI, Microfluidics, MACS, Advanced culture media, Oocyte vitrification, Assisted hatching, Time Lapse imaging, spindle view and Electronic Witnessing. The most common practice suggestions were tabulated and identified.
Main results and the role of chance: The survey reports huge need for training for different add on treatments (SDF -91.4%, IMSI - 81.2%, PICSI- 66.5%, Microfluidics- 55.9%, MACS -55.3%, MicroTese- 86.9%, using advanced culture medias{Calcium ionophore- 73.4%, Hyaluronan rich media- 52.1%, growth cytokine rich media-48.5%, Theophylline for sperm motility-50.9%},oocyte Vitrification 85.5%, Assisted Hatching 75.4%, Time-lapse and Electronic witnessing 77.3%, Polarised microscopy for spindle assessment 73.5%). The Most preferred mode of training for more invasive procedures was Hands on training, followed by On the job training and validation followed by workshops(SDF-62.6%, IMSI- 61%,PICSI-56.1%, MACS and Microfluidics 38.8%,microTese-50.6%,Oocyte freezing 85.5%, assisted hatching 67.8%,Time-lapse and electronic witnessing 77.3%, Spindle view 73.5%). The most preferred mode of training for non invasive procedures was Workshops and Observerships, followed by CME's, followed by product Leaflets(44.4%,42.1%,13.5% respectively). The most common answer for the disadvantages of not being trained was unable to use the technology to its fullest potential(88.5%), whereas the most common answer for the benefit of being trained was better outcomes with said technologies(76.6%).
Limitations, reasons for caution: This study includes responses from embryologists who have varied levels of experience, while the need for training can be established based on these results, a junior level embryologist might answer the survey differently as compared to a senior or a lab director.
Wider implications of the findings: This is a first of its kind large survey, suggesting the need for training and validation from the perspective of the embryologist. This data can be used in formulating guidelines for future trainings and can help regulators in deciding on the most preferred mode of training.
Trial registration number: -

P-772 Blastocyst quality and perinatal outcomes in women undergoing single blastocyst transfer in frozen cycles

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Study question: Is the morphological grading systems of a blastocyst associated with perinatal outcomes in women undergoing frozen-thawed single blastocyst transfer (SBT)?

Summary answer: Preferential transfer of a blastocyst based on their morphological grading systems appears to be supported by observed differences in perinatal outcomes.
What is known already: The transfer of a morphologically good quality blastocyst is associated with higher implantation and pregnancy rates as compared with a poor quality blastocyst. However to date, the association of the morphologic parameters of the blastocyst (developmental stage, inner cell mass (ICM), and trophectoderm (TE)) with the perinatal outcomes after blastocyst transfer remains unknown.
Study design, size, duration: A retrospective cohort study including 21,648 frozen-thawed SBT cycles from January 2013 to March 2019.
Participants/materials, setting, methods: 6037 women with singleton delivery in Peking University Third Hospital were eligible for analysis. Multivariate logistic regression was used to test the risk of factors with the expression of crude odds ratios (OR) and adjusted OR (aOR) with 95% confidence intervals (CI).
Main results and the role of chance: Transfer of a blastocyst with grading lower than 3BB was associated with a higher chance of female baby (49% vs 43%, aOR = 1.27 (1.12, 1.43)) and a higher rate of cesarian section (C-section) (69% vs 65%, aOR = 1.17 (1.03, 1.34)). Compared with stage 4 blastocyst, transfer of a stage 3 blastocyst was associated with a higher chance of preterm delivery (PTD) (aOR = 1.77 (1.08, 2.90)). Both stage 3 and stage 6 blastocyst transfer was associated with a lower chance of female baby (aOR = 0.68 (0.48, 0.97), 0.66 (0.47, 0.93), respectively). Compared with grade A ICM blastocyst transfer, Grade B ICM and grade C ICM blastocyst transfer was associated with a lower chance of a female baby (adjusted OR = 0.84 (0.73, 0.96), 0.63 (0.48, 0.83), respectively) and a higher risk of large for gestational age (LGA) (aOR = 1.20 (1.01, 1.42), 1.46 (1.07, 1.98), respectively). Grade C ICM blastocyst transfer was associated with an increased risk of macrosomia (aOR = 1.66 (1.14, 2.42)).Grade B TE and grade C TE blastocyst transfer had a lower risk of gestational diabetes mellitus (GDM) (aOR = 0.76 (0.60, 0.98), 0.69 (0.50, 0.94), respectively) than grade A TE blastocyst transfer.
Limitations, reasons for caution: The main limitations of this study were its retrospective nature and the relative subjectivity of blastocyst scoring. The follow-up was conducted through a phone call and some patients might not report their obstetrical and neonatal outcomes, leading to a relatively lower rate of several obstetrical outcomes.
Wider implications of the findings: Transfer of a poor quality blastocyst is associated with a higher rate of C-section. The association between ICM grading and LGA and macrosomia would suggest that blastocysts with grade A ICM grading should be transferred preferentially and supports the use of current morphological grading systems for embryo prioritisation.
Trial registration number: N/A

P-773 Infertility treatment and the risk of small for gestational age births: a population-based study in the United States

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Study question: What is the association between infertility treatments and small for gestational age (SGA) births?
Summary answer: Women who conceived pregnancies with any infertility treatment had a decreased risk of SGA <10th, <5th and <3rd percentiles compared to naturally conceived pregnancies.
What is known already: Assisted reproductive technology (ART) and other infertility treatments have long been associated with an increased risk of SGA births, which confers a greater risk of perinatal morbidity and mortality compared to appropriate for gestational age births.
Study design, size, duration: This is a cross-sectional study of 16,836,228 births in the United States (US) between 2015-2019. The exposure group included women who underwent any infertility treatment, including ART and prescribed fertility enhancing medications. The comparison group included those who had naturally conceived pregnancies. The primary outcome was SGA birth, defined as sex-specific birthweight <10th percentile for gestational age. Secondary outcomes included SGA <5th and <3rd percentile births.
Participants/materials, setting, methods: Pregnant subjects (n= 16,836,228) in the US who delivered non-malformed, singleton live births between 24-44 weeks' gestational age. We estimated risk of SGA births in relation to any infertility treatment from fitting log-linear Poisson regression models with robust variance. Risk ratios (RR) and 95% confidence intervals (CI) were estimated as the effect measure before and after adjusting for confounders. We also performed a sensitivity analysis to correct for potential non-differential exposure misclassification and unmeasured confounding biases.
Main results and the role of chance: During the study period, 1.4% (n=231,177) of non-malformed singleton live births resulted from infertility treatments (0.8% ART and 0.6% fertility enhancing medications). Of these, 9.4% (n=21,771) of pregnancies conceived with infertility treatment were complicated by SGA <10th percentile compared to 11.9% (n=1,755,925) of naturally conceived pregnancies. For pregnancies conceived with infertility treatment versus naturally conceived pregnancies, the adjusted RR for SGA <10th percentile was 1.07 (95% CI 1.06, 1.08). However, after correction for misclassification bias and unmeasured confounding, infertility treatment was found to be protective for SGA and conferred a 27% reduced risk of SGA <10th percentile (bias-corrected RR 0.73, 95% CI 0.53, 0.85). These trends were similar for analyses stratified by exposure to ART and fertility enhancing medications and secondary SGA outcomes, including SGA <5th and <3rd percentile.
Limitations, reasons for caution: All information collected on infertility treatment relies on self-reporting by patients and recording by hospital staff at the time of delivery, which likely resulted in underreporting of infertility treatments. Additionally, we cannot determine the impact of interventions that were not recorded, such as intrauterine insemination (IUI).
Wider implications of the findings: Compared to naturally conceived pregnancies, exposure to infertility treatment is associated with reduction in the risk of SGA births. These findings, which are contrary to some published reports, likely reflect changes in the modern practice of infertility care in the US, and importantly, robust analysis of the national data.
Trial registration number: not applicable

P-774 Clinical effectiveness of elective single versus double blastocyst transfer in women aged 36 years or older

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Study question: If the elective single-blastocyst transfer (eSBT) strategy can be applied to women aged 36 or older.
Summary answer: In women ≥36 years old with at least two blastocysts, eSBT increased cumulative livebirth rate (LBR) while minimizing twins compared with double blastocyst transfer (DBT). What is known already: In young women with a good prognosis, eSBT policy is an accepted strategy to maintain LBR while decreasing multiple gestation. However, in many areas of the world DBT is still applied in older women.
Study design, size, duration: We performed a retrospective cohort study of 429 women aged ≥36 years or older who received IVF ovarian stimulation cycles between Jan 2015 and Oct 2018 and who had at least two blastocysts. Women were followed up until Oct 2020 for their fertility outcomes including cumulative live birth and multiple pregnancies. The study was performed at the Northwest Women and Children's Hospital, Xi'an, China.
Participants/materials, setting, methods: Out of 429 women, 240 underwent a fresh cycle of eSBT and 189 DBT. The subsequent frozen-thawed embryo transfer cycles were a combination of single- and double- blastocyst

transfers, more commonly the latter. Analysis was stratified for patients in age groups 36-37, 38-39 and ≥40 and quality of the blastocyst transferred, as graded by morphological examination. Outcomes were the LBR in the fresh cycle, cumulative LBR and multiple rate after fresh and frozen embryo transfers.

Main results and the role of chance: The cumulative LBR was 74.2% (178/240) for eSBT versus 63.0% (119/189) for DBT (OR=1.69, 95%CI 1.12-2.56), irrespective of female age. The multiple rate was 9% (16/178) after eSBT versus 29.4% (35/119) after DBT (*P*- value < .001). The total number of children born was 194 after eSBT versus 154 after DBT. Stratified by female age, the cumulative LBRs in women aged 36-37 (78.9 vs 70.5%), 38-39 (68.9 and 61.1%) and ≥ 40 years (59.3 and 47.5%), were higher after eSBT compared with DBT, however, the differences did not reach statistical significance in each subgroups. LBRs in the fresh cycles were comparable for patients with eSBT compared with DBT (52.1% vs. 52.4%, OR=0.99, 95%CI 0.68-1.45). In women < 40 years, DBT resulted in a small non-significant increase in LBR in the fresh transfer (63.2% vs. 61.2%, 95%CI=0.64-1.85, 36-37 years; 48.1% vs. 41.0%, 95%CI=0.64-2.80, 38-39 years) at the expense of a marked increase in twinning rate (0-5.4% vs. 31.7-34.6%). For women ≥40 years, no significant differences were observed in the LBR (37.0% vs 45%, 95%CI 0.47-4.07) or twinning rate (0 vs 7.7%) between eSBT and DBT group. The findings persisted with and without accounting for quality of the blastocyst transferred.

Limitations, reasons for caution: This study is limited by its observational character.

Wider implications of the findings: In women ≥36 years with two blastocysts, eSBT should be the preferred treatment which maximizes the cumulative LBR for a decrease in the rate of multiple pregnancies.

Trial registration number: Not applicable

P-775 Obstetric and perinatal outcomes of singleton pregnancies after blastocyst-stage embryo transfer: a systematic review and cumulative meta-analysis

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Study question: Are obstetric/perinatal outcomes different in singleton pregnancies following blastocyst-stage embryo transfer when compared to cleavage-stage embryo transfer and have results changed over time?

Summary answer: Pregnancies following blastocyst are consistently associated with higher risk of large for gestational age and lower risk of small for gestational age babies

What is known already: Extended embryo culture to blastocyst-stage is widely used to select best embryos in in vitro fertilisation (IVF) cycles to improve pregnancy rates. Transfer of blastocyst-stage embryos is increasing with this being the default strategy in most clinics. As blastocysts are kept in culture until day 5, 6 or 7 after oocyte fertilisation, there are suggestions that longer exposure to culture media may have a negative impact on pregnancy outcomes. More recent primary studies have challenged some of the initial findings. We therefore conducted an updated systematic review and cumulative meta-analysis (CMA) to examine if these results have changed over time.

Study design, size, duration: Systematic review of studies published between 1980 and 2020, followed by aggregated meta-analysis and CMA to track the accumulation of evidence over the period of time. Exposed group: singleton pregnancies following blastocyst transfer. Non-exposed group: singleton pregnancies following cleavage-stage transfer. Sub-group analyses were conducted on fresh and frozen-thawed embryo transfers. Perinatal (categories of preterm birth and birth weight) and obstetric outcomes (hypertensive disorders of pregnancy, gestational diabetes, c-section, placental anomalies) were compared between the groups.

Participants/materials, setting, methods: Medline, EMBASE, CINHAL, Web of Science, Cochrane Central Register of Clinical Trials and International Clinical Trials Registry Platform databases were searched. Relevant journals were searched for advance access publications. Critical Appraisal Skills Programme (CASP) checklists were used to assess study quality. Two independent reviewers extracted data in 2 × 2 tables. Aggregated and CMA were performed using Comprehensive Meta-Analysis software. Risk ratio (RR) with 95% confidence interval (CI) were calculated.

Main results and the role of chance: A total of 33 observational studies were included (n = 574,756 singleton pregnancies). Pregnancies following blastocyst-stage embryo transfer are associated with a higher risk of preterm birth (PTB) (RR 1.09; 95% CI 1.01–1.17), very preterm birth (VPTB) (RR 1.15; 95% CI 1.07–1.24), large for gestational age (LGA) babies (RR 1.13; 95% CI 1.08–1.19), c-section (RR 1.05; 95% CI 1.02–1.09), and with a lower risk of small for gestational age (SGA) babies (RR 0.86; 95% CI 0.81–0.93) as compared to singleton pregnancies following cleavage-stage embryo transfer.

These findings were maintained in both fresh and frozen-thawed sub-groups for LGA and SGA. PTB was not significantly different in both sub-group analyses. The risk of VPTB was higher after blastocyst-stage embryo transfer only in the sub-group analysis of fresh embryo transfers (RR 1.17; 95% CI 1.09–1.27) and that of c-section only in the frozen-thawed sub-group (RR 1.08; 95% CI 1.04–1.12).

No other statistically significant differences for the other outcomes were noted.

The CMA suggests that for SGA and LGA subsequent studies have increased the precision of the point estimate with no change in the direction or magnitude of the treatment effect since 2014.

Limitations, reasons for caution: This analysis was constrained by the intrinsic limitations of observational studies with some of them receiving a CASP score < 10. Adjustment for confounders was not possible and a high degree of clinical and statistical heterogeneity was noted among studies.

Wider implications of the findings: Blastocyst is associated with a higher risk of LGA and a lower risk of SGA with a stable body of evidence since 2014. We may need to revisit the default position of extending embryo culture and individualise care, until further high-quality data from individual-patient-data of large registries are available.

Trial registration number: Not applicable

P-776 Singleton pregnancies conceived with infertility treatments and the risk of neonatal and infant mortality

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Study question: Is maternal infertility treatment associated with an increased risk of neonatal and infant mortality when compared to natural conception?

Summary answer: Infertility treatment is associated with a 70% increased adjusted risk of neonatal mortality. This association is strongly mediated by preterm delivery.

What is known already: The number of assisted reproduction technology (ART) cycles performed in the United States (US) increased by 39% from 142,435 cycles in 2007 to 197,737 in 2016. Within this growing experience, several studies described an increased risk of preterm delivery, low birth weight, congenital malformations, neonatal intensive care unit admission, stillbirth, and perinatal mortality among singletons conceived through ART compared to those conceived naturally. Experts have called for ART patients to be advised of potential increased risk for adverse perinatal outcomes and for obstetricians to manage these pregnancies as high risk.

Study design, size, duration: This is a cross-sectional study of 11,289,466 pregnancies in the United States (US) from 2015-2017 that resulted in a non-malformed singleton live birth. The exposure group includes births resulting from any infertility treatment method, including ART and fertility-enhancing drugs. The control group includes births resulting from natural conceptions. The primary outcomes measured were neonatal (within 1 month), post-neonatal (1 month to a year), and infant (up to 1 year) mortality.

Participants/materials, setting, methods: Pregnancies (n=11,289,466) resulting in a non-malformed singleton live birth in the US from 2015-2017. Associations were estimated from log-linear Poisson regression models with robust variance. Risk ratio (RR) and 95% confidence interval (CI) were derived as the effect measure with adjustments for confounders. The impact of exposure misclassification and unmeasured confounding biases were assessed. A causal mediation analysis of the infertility treatment-mortality association with preterm delivery (<37 weeks) was performed.

Main results and the role of chance: Any infertility treatment was documented in 1.3% (n=142,215) of singleton live births during the study period. Any infertility treatment was associated with a 70% increased adjusted risk of neonatal mortality (RR 1.70, 95% CI 1.54-1.88), with an even higher risk for early neonatal (RR 1.82, 95% CI 1.63-2.05) than late neonatal (RR 1.37, 95% CI 1.11-1.69) mortality. These risks were similar among pregnancies conceived through ART and treatment with fertility-enhancing drugs. The mediation analysis showed that 68% (95% CI 59-81) of the total effect of infertility treatment on neonatal mortality was mediated through preterm delivery. In a sensitivity analysis, following corrections for exposure misclassification and unmeasured confounding biases, these risks were higher for early neonatal (bias-corrected RR [RRbc] 2.94 95% CIbc 2.16-4.01), but not for late neonatal (RRbc 1.04, 95% CIbc 0.68-1.59) mortality.
Limitations, reasons for caution: Limitations of the study include the potential underreporting of infertility treatment on birth certificates and potential confounding from sociodemographic characteristics that were not accounted for in this study.
Wider implications of the findings: Pregnancies conceived with infertility treatment are associated with increased neonatal mortality and this association is mediated by the increased risk of preterm delivery. Knowledge of this risk should be shared with prospective couples consulting for fertility care in order to best provide adequate informed consent.
Trial registration number: not applicable

P-777 Comparison of GnRH-a trigger and GnRH-a plus low-dose HCG trigger for high ovarian responders in IVF/ICSI: A retrospective study based on propensity score matching

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Study question: Does GnRH agonist trigger for high responders during IVF/ICSI cycles improve the number of good-quality embryos, the incidence of moderate-to-severe OHSS, and pregnancy outcome compared to GnRH-a plus low-dose HCG?
Summary answer: GnRH-a trigger alone can effectively reduce the incidence of moderate-to-severe OHSS in women with high ovarian responses without affecting embryo quality.
What is known already: Previous studies have shown conflicting results on the different trigger protocol in high responders in IVF/ICSI outcomes, and as for women with high ovarian response, there is little known about the effects of GnRH-a plus low-dose HCG versus GnRH-a alone on oocytes maturation, the rate of good quality embryos, the incidence of moderate-to-severe OHSS, and pregnancy outcome during IVF/ICSI cycles.
Study design, size, duration: A retrospective analysis was conducted on patients with high ovarian response who received IVF/ICSI treatment with a flexible GnRH antagonist regimen, at the Center of Reproductive Medicine, Chengdu Jinjiang Hospital for Maternal and Child Health Care, from January 1 2017 to December 31 2018. Using 1:1 propensity score matching, 513 cases entered each group (a total of 1,026 females).
Participants/materials, setting, methods: The high responders were included and assigned to groups A (0.2 mg triptorelin) and B (0.2 mg triptorelin plus 2000 IU HCG) for final oocyte maturation. Their basic clinical characteristics, information about controlled ovarian stimulation cycle, embryologic data, and pregnancy outcome in FET were retrospectively compared. The main outcome measures of the study were the rate of good-quality embryos, the number of available embryos, the incidence of moderate-to-severe OHSS, and the cumulative live-birth rate.
Main results and the role of chance: Using 1:1 propensity score matching, 513 females were included in each group. No significant differences in baseline clinical data were found between the two groups, including age at diagnosis, spouse's age, the duration of infertility, the infertility type, and the cause of infertility, BMI, anti-Müllerian hormone (AMH) levels, and the antral follicle count (AFC) ($p > 0.05$). None significant differences were found in the total doses of gonadotropin (Gn), the duration of ovarian stimulation, serum P and LH levels on the trigger day, the number of oocytes retrieved, the rate of 2PN embryos, and the rate of good-quality embryos ($p > 0.05$). The serum E2 level on the trigger day in group A was significantly higher than that in group B ($p < 0.001$). Women in group A had a lower incidence rate of moderate-to-severe OHSS than individuals in group B ($p < 0.001$). There was a non-significant difference in the cumulative live-birth rate between the two groups ($p > 0.05$).
Limitations, reasons for caution: As this is a retrospective study that uses data initially collected for other purposes, limitations may exist in the selection, implementation, and measurement biases that cannot be avoided. However, our study underlies the need for further prospective, multi-center joint-controlled studies to validate these findings.
Wider implications of the findings: This study demonstrates that GnRH-a alone can reduce the incidence of moderate-to-severe OHSS without harming embryo quality in women with high ovarian response. These findings need further prospective validations in hyperresponsive populations by multi-center, large-sample, randomized controlled studies.
Trial registration number: N/A

P-778 Repeated cryopreservation process impairs embryo implantation potential but does not affect neonatal outcomes

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Study question: Does repeated cryopreservation process effect embryo implantation potential and neonatal outcomes of human embryos?
Summary answer: Repeated cryopreservation impaired embryo implantation potential, resulting in a lower live birth rate and higher miscarriage rate, despite a comparable neonatal complication rate.
What is known already: With significant advances in the field of ART, the number of available embryos for transfer per cycle has also increased, resulting in a slew of surplus embryo cryopreservation. However, limited researches have focused on the embryonic development potential, clinical outcomes, pregnancy complications as well as the neonatal complications of embryos experiencing repeated cryopreservation.
Study design, size, duration: This was a retrospective, single-center cohort study. All ART cycles from January 2014 to December 2018. Age, body mass index, and number of oocytes retrieved were preferentially matched within a required range, with a total of 709 couples included in the study.
Participants/materials, setting, methods: The study was conducted in the Reproductive Medicine Centre affiliated to a university. Preferentially matched participants were divided into three groups according to the times of embryo cryopreservation: the fresh group (n=249), the cryopreservation group (n=244) and the re-cryopreservation group (n=216). Embryo implantation rate, live birth rate, miscarriage rate, and neonatal complication rate were compared among these three groups.
Main results and the role of chance: The embryo implantation rate, clinical pregnancy rate and live birth rate in the re-cryopreservation group were significantly lower, and there was also a slight increase in the miscarriage rate. Logistic regression analysis indicated that embryos with repeated cryopreservation and lower TE scores were at higher risk of embryo implantation failure in single embryo transfer cycles (OR=1.79 and 1.56 respectively). No significant differences were observed in gender, gestational age, birthweight, neonatal abnormality, and neonatal complications among the groups.
Limitations, reasons for caution: This was a retrospective cohort study conducted in single center. A multi-center prospective study with a larger sample size in well-matched participants is needed to reinforce our findings.
Wider implications of the findings: Our findings demonstrated the adverse effect of repeated cryopreservation on embryo implantation potential. To avoid embryo waste, or in some special circumstances such as re-biopsy in PGT cycles, an additional cryopreservation on embryos was considered to be available to achieve clinical pregnancy and live birth.
Trial registration number: not applicable

P-779 Vascular stiffness in children born after embryo transfer at 8-9 years of age

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Study question: Do 8-9-year-old singletons conceived after frozen (FET) or fresh embryo transfer (Fresh ET) have increased vascular stiffness compared to naturally conceived (NC) children?
Summary answer: FET and Fresh ET was not associated with increased vascular stiffness or altered cardiovascular autonomic reflexes as compared to NC children.
What is known already: Normally, vascular stiffness increases during childhood, and in adults with the metabolic syndrome increased vascular stiffness is associated with symptomatic cardiovascular disease. Children conceived after FET and Fresh ET are at risk of being large- and small-for-gestational-age, respectively. Epigenetic modulation during assisted reproductive technologies (ART) has been suggested to influence cardiovascular risk factors, and previous studies have shown that children conceived after ART are at increased risk of insulin resistance, endothelial dysfunction and increased arterial blood pressure. It is not known if ART procedures alter vascular stiffness of children.
Study design, size, duration: In a cohort study including 8-9 years old singletons conceived after FET, Fresh ET and NC (50 in each group), we used cardiac magnetic resonance imaging (CMR) and cardiovascular autonomic reflex testing (CART) to compare arterial stiffness. The study was powered to detect a difference between groups of aortic distensibility from 8.9 to 8.0, comparable to what is seen in a 5-year older cohort of children (beta 0.80, alpha 0.05). Inclusion period 18 months.
Participants/materials, setting, methods: Singletons were identified through the Danish IVF Registry and the Medical Birth Registry. NC children were matched by sex and birth year with FET children. Exclusion criteria were congenital heart disease, maternal preeclampsia, gestational diabetes or diabetes mellitus. Artery stiffness was assessed from blood pressure and aortic distensibility, pulse wave velocity (PWV), cardiac output and total peripheral resistance by CMR. CART was investigated non-invasively in 40 children. Measurements were performed blinded to the child group.
Main results and the role of chance: Maternal age at delivery was higher in the FET (42.5±5.5 years) and Fresh ET (40.5±6.1 years) compared to the NC group (38.2±5.7 years). In the ART groups, mothers were more likely to have a high educational level (FET 50% and Fresh ET 56.2%) compared to mothers in the NC group (30.6%) (both ANOVA-p<0.05). As expected, children conceived after FET had a higher birth weight standard-deviation-score (0.4±1.1+) compared to Fresh ET (-0.1±1.0) and NC (-0.2±1.1). Among study groups, no significant differences were observed in systolic and diastolic blood pressure (FET 109±6/64±6 mmHg; Fresh ET 109±7/65±5 mmHg; NC 108±8/65±5 mmHg; ANOVA-p>0.05). Heart rate was also similar in all study groups (FET 79±12 bpm; Fresh ET 79±9 bpm; NC 78±11 bpm; ANOVA-p>0.05). No significant differences were observed between groups in total aortic PWV (FET 3.69±0.75 m/s; Fresh ET 3.49±0.31 m/s; NC 3.59±0.61 m/s; ANOVA-p>0.05). Aorta ascendens distensibility was similar in study groups (FET 11.12±3.55 10-3mmHg-1; Fresh ET 11.77±2.97 10-3mmHg-1; NC 11.43±2.82 10-3mmHg-1, ANOVA-p>0.05). Furthermore, distensibility of aorta descendens and aorta abdominalis, PWV of arcus aorta and PWV from aorta descendens to abdominalis, cardiac output, total peripheral resistance and CART were similar in study groups. Outcome variables remained non-significant after adjustment for potential confounders.
Limitations, reasons for caution: The participation rate was higher in the ART groups (FET 40% and Fresh ET 32%) compared to NC (17%) and hence a selection bias is possible. Data from CART should be interpreted cautiously due to lower number of participating children in these tests.
Wider implications of the findings: Our study did not find any associations between FET or Fresh ET compared to NC children and arterial stiffness. Nor, any associations to CART could be made. Further studies are needed in younger adults to better exclude important long-term effects of ART.
Trial registration number: NCT03719703

P-780 The increase of single embryo transfers does not impair pregnancy and live birth rates, although it lowers twin rate

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Study question: How does significant increase of the proportion of single embryo transfer over 10 years period affect pregnancy, live birth and twin rate.
Summary answer: Increase in single embryo transfer doesn't change pregnancy and live birth rate, although it significantly lowers twin rate.
What is known already: Due to widely used approach in IVF of transferring multiple embryos to improve the pregnancy and birth rate, multiple pregnancies, mostly twin, are quite common. But because they are more often associated with adverse neonatal and perinatal outcomes as singleton pregnancies, they are not desirable. Therefore, more and more often the transfer of single embryo is encouraged. Furthermore, in the last years with improvements in cryopreservation techniques leading to effective cryopreservation of supernumerary embryos, there is more options for performing repeated single embryo transfers.
Study design, size, duration: We retrospectively collected the data of all fresh embryo transfers in couples treated in our centre from January 2010 to December 2019. We excluded embryo transfer where embryos were derived from cryopreserved oocytes and analysed the outcome of fresh embryo transfer regarding to the number of transferred embryos.
Participants/materials, setting, methods: In our analysis we included 10583 fresh embryo transfers. We tried to evaluate how the proportion of single embryo transfer has changed through analysed period of time and if this led to any differences in pregnancy, live birth, and twin rate. To determine the differences between the groups, the data were analysed with one-way ANOVA and Pearson's chi-square, as appropriate. Statistical significance was set at P<0.05.
Main results and the role of chance: The analysis revealed that the proportion of single embryo transfers significantly increased from year 2010 to year 2019 (from 28% to 73%; P<0.001). The proportion increased every year, minimum increase was 1% whereas maximum increase was 16%. This increase over the years did not negatively affect the pregnancy (32% in 2010 vs. 34% in 2019: p=0.317) and live birth rates (24% vs. 25%; p=0.584), although it had favorable effect on twin rate (16% vs. 7%; p=0.002). If we separately analyzed only single and double embryo transfer, we observed that pregnancy (24% in 2010 and 34% in 2019; p=0.001) and live birth rates (17% vs. 26%, p=0.001) significantly increased after single embryo transfers, but no difference was observed in double embryo transfers (pregnancy rate: 35% vs. 35%, p=1; live birth rate: 27% vs. 22%, p=0.097; twins rate: 20% vs. 27%, p=0.244). Additionally, we observed that female mean age value significantly increased over analyzed period (34.2±4.5 years in 2010 vs. 35.7±4.7 years in 2019, p<0.001), although there was no difference in mean number of retrieved oocytes (8.2±5.4 vs. 8.1±4.9, p=1) and obtained embryos (4.5±3.3 vs. 4.2±2.9; p=0.684).
Limitations, reasons for caution: The limitation of the study is retrospective design, and not evaluating the influence of elective single embryo transfer. Also, the IVF laboratory methods and IVF culture media improved over the years meaning they could be partly responsible for observed differences.
Wider implications of the findings: Single embryo transfer could probably be performed in even higher proportion without lowering the chances for pregnancy.
Trial registration number: not applicable'

P-781 Birthweight is not affected by freezing process. Results from a quasi-experimental study using the Oocyte Donation Model

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Study question: Is the freezing process responsible to increase the birthweight or the incidence of Large for Gestational Age (LGA) in Frozen Embryo Transfers (FET)?
Summary answer: Neither the birthweight nor the LGA incidence were different in embryos that underwent the freezing-thawing process.
What is known already: Freezing-thawing constitutes one of the processes with a potential impact on the health of the newborn. Data coming from register-based studies and metaanalisis have found an increase in birthweight with a higher incidence of LGA in newborns coming from FET. This is a matter of concern since epigenetic alterations have been suggested to explain this finding casting doubts on future health during childhood and adulthood. Clarifying the safety of cryotechniques should be a priority taken into account that at present frozen embryo transfers outnumber fresh embryo transfers in IVF clinics.
Study design, size, duration: This retrospective cohort study evaluated 670 women oocyte recipients who underwent fresh (367 cycles) or frozen embryo transfer (303 cycles) at Instituto Bernabeu between July 2017 and March 2019. All recipients were prepared with substitutive cycle and received single blastocyst embryo transfers on day five. All of them at the same culture medium, resulting in a singleton live birth.
Participants/materials, setting, methods: 1637 patients were assessed for eligibility but 967 were excluded. The sample size has been calculated accepting an alpha risk of 5% and a beta risk of 20%. A sample size of 266 patients (133 per group) is required to detect a minimum mean difference of 275 grams with a standard deviation of 800 grams. Pearson´s Chi-square test (univariate) and binary logistic regression (multivariate for confounding factors) were used to analyze association between variables.
Main results and the role of chance: Maternal age (42.21 ± 4.45; 42.79 ± 3.83 p=0.519), BMI (23.34 ± 3.69; 24.99 ± 15.52; p=0.060), maternal parity (Nulliparous 81.5%; 85.5%; Multiparous 18.5%; 14.5% p= 0.177), gestational diabetes (4.9%; 4.3% p=0.854), preeclampsia (2.7%; 5.6% p=0.074), hypertensive disorders (3.3%; 2.3% p=0.494), maternal smoking (10.8%; 13.0% p=0.475), gestational age (38.96 ± 1.97; 38.77 ± 2.15; p=0.207) and liveborn gender (Female 44.5%; 48.8%; Male 55.5%; 51.2%p=0.276) do not present statistically significant differences between fresh or frozen groups, respectively.

However endometrial thickness was statistically signiticantly different in both groups (8.83mm ± 1.73 fresh; 8.57mm ± 1.59 frozen p=0.035)

The mean birthweight did not present statistically significant differences (3239.21 ± 550.43 fresh; 3224.56 ± 570.83 frozen p=0.211). There were also no differences regarding macrosomy (7.1% fresh; 6.3% frozen p=0.317), LGA (6.0% fresh; 6.7% frozen p=0.866), pre-term birth (10.9% fresh; 9.0% frozen p=0.988), very pre-term birth (0.8% fresh; 1.3% frozen p=0.999), and extremely pre-term birth (0% fresh; 1.0% frozen p=0.998).

There were statistically significant differences regarding underweight (10.0% fresh; 7.0% frozen p=0.020), but there were no differences in very low weight (0.6 fresh; 1.1% frozen p=0.972) and SGA (1.9% fresh; 0.7% frozen p=0.432).
Limitations, reasons for caution: Despite a quasi-experimental design, the synchronization in fresh embryo transfer drove to a longer preparation with a thicker endometrium. It's not possible to rule-out the influence in the results of this parameter.
Wider implications of the findings: As a hypothesis, the increase in birthweight and/or an abnormal placentation in FET could be explained by the endometrial preparation more than the freezing process. Studies must be planned in the future to explore the possibility of changes in the birthweight between embryos transferred in natural vs artificial endometrial preparations.
Trial registration number: Not applicable

P-782 A natural language processing approach of global survey results on what the embryologist thinks and faces.

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Study question: What are the major problems faced by embryologists at 1) Clinic level, 2) Professional level, 3) Personal level, and 4) What are their career goals?
Summary answer: Embryologists, essential professionals of Fertility Centres, are less satisfied in many quantifiable aspects, but they love their profession and have many aspirational goals.
What is known already: IVF success depends in part on embryologists' skills. The need to recognize clinical embryology as a specialty and clinical embryologists' educational level, responsibilities, and workload have been addressed by a few national societies. However, data are lacking from the embryologists' viewpoint at a global level about their profession. Qualitative data-analysis methods provide thick, rich descriptions of subjects' thoughts, feelings, and lived experiences but can be time-consuming, labor-intensive, and prone to bias.
Study design, size, duration: A questionnaire was prepared using SurveyMonkey online software (SurveyMonkey, Inc., USA) and distributed to IVF lab professionals through embryology societies, online social media, and email databases. The questionnaire consisted of open-ended questions focused on identifying problems faced by embryologists at the clinic, in the profession, and in a personal level, as well as questions about their career outlook. The survey was active from May 2016 until February 2017. From 73 countries, 720 responses were obtained.
Participants/materials, setting, methods: Using natural language processing (NLP), the top 15 most frequently used keywords were identified and correlated with each other. Stronger correlation (≥0.5) between semantically similar words expressing a strong signal from each answer, and their usage was further analyzed for positive versus negative sentiment. By normalizing the frequency of positive/negative samples for each keyword as a percentage, "sentiment wheels" were produced, identifying the key concepts that respondents answered and quantifying how they felt about them.
Main results and the role of chance: The responses received were from 80% private, 17% public and 3% other ART settings distributed all over the world. From the embryologists' viewpoints reported and after the NLP processing it was shown that the common topics related to strong negative sentiments were: embryologists' remuneration (0.6) at the Clinic level; certification (0.7), recognition (0.5), respect (0.5), learn (0.5) and experience (0.5) at the Professional level; and remuneration (0.7), emotional (0.5) dealing (0.5) at the Personal level. Renumeration was reported and strongly related to embryologists' viewpoint at both the clinic and personal level in combination with the need for certification, recognition and ongoing development at the Professional level. Moreover, the NLP processing demonstrated that the common topics on career goal analysis related to strong positive sentiments were: teaching (0.7), education (0.7), and continuation (0.5) all three topics are compatible with a professional orientation open to ongoing development and practice advancement. The NLP and the manual data analysis project an image of the typical embryologist as a knowledge seeking professional who is deeply dedicated to the job but feels the need for professional development and suffers some lack of recognition and feels in some cases not fairly treated as an employee.
Limitations, reasons for caution: The data obtained is limited. Only one natural language processing model was used to analyze the results. Different analysts using other methods may have different results. For these reasons, the results should be interpreted with caution.
Wider implications of the findings: It is important to focus on the lab as an organization and not just a service for the patients in treatment at the moment. The NLP results ultimately obtained may help streamline professional satisfaction efforts, and guide future quality management strategies
Trial registration number: not applicable

P-783 Clinical, obstetric and perinatal outcomes after vitrified-warmed euploid blastocyst transfer are independent of cryostorage duration

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Study question: Is cryo-storage duration associated with the outcomes after vitrified-warmed euploid single blastocyst transfer?
Summary answer: Lower live-birth-rates from blastocysts cryo-stored for periods longer than 3-months are mostly imputable to the worse quality of the embryos being warmed across sequential transfers.
What is known already: Blastocyst vitrification is crucial in modern IVF. Given its widespread application, a constant comprehensive monitoring of its effect on reproductive outcomes is pivotal. For instance, the effect of cryo-storage duration on embryo implantation potential, gestational and perinatal outcomes is object of a still ongoing investigation. The evidence in this regard are contrasting especially with regard to similar or decreased live birth rates among blastocysts subject to long-term cryo-storage. When investigating the neonatal outcomes, instead, no impact of blastocyst cryo-storage duration has ever been reported to date. Yet, data on euploid blastocysts and adjusted for quality and full-blastulation day are needed.
Study design, size, duration: Retrospective observational study. We included 2688 vitrified-warmed euploid single blastocyst transfers. The primary outcome was the live-birth-rates (LBR) according to cryo-storage duration clustered as ≤60, 61-90, 91-180, 181-360, 361-720, 721-1080 and >1080-days. The secondary outcomes were the miscarriage rate, the rates of gestational and perinatal issues among the deliveries, and the mean gestational age and birthweight among the babies born. All data were adjusted for confounders through linear or logistic regression analyses. Participants/materials, setting, methods: We included all vitrified-warmed transfers (range:1-8) conducted between May-2013 and March-2020 by 1884 patients (age:38±3yr) undergoing one blastocyst stage PGT-A cycle and obtaining ≥1 euploid embryo at our private clinic. Among putative confounders, only the number of sequential transfer from the same patient, blastocyst quality (Gardner's scheme) and full-blastulation day (5-7) significantly associated with the LBR through univariate regressions. No association was reported for sperm factor, maternal age, incubator, and culture media.
Main results and the role of chance: The LBR of euploid blastocysts cryo-stored for ≤60-days was 49.4% (N=319/646) versus 48.7% (N=292/599; OR:0.98,95%CI:0.78-1.21,p=0.82) between 61-90-days, 42.9% (N=291/679; OR:0.77,95%CI:0.62-0.96,p=0.02) between 91-180-days, 41.7% (N=169/405; OR:0.73,95%CI:0.57-0.94,p=0.02) between 181-360-days, 34.7% (N=50/144; OR:0.55,95%CI:0.37-0.79,p<0.01) between 361-720-days, 53.4% (N=63/118; OR:1.17,95%CI:0.79-1.74,p=0.42) between 721-1080-days, and 50.5% (N=49/97; OR:1.05,95%CI:0.68-1.60,p=0.83) for >1080-days. However, when these data were adjusted for blastocyst quality and full-blastulation day, all the multivariate-OR were not-significant. Indeed, the longer the cryo-storage period the worse the quality of the euploid blastocysts transferred (e.g. AA-blastocysts were 74% among embryos cryo-stored for ≤90-days, but always <70% for embryos cryo-stored for longer periods, p<0.01; similarly, day5-blastocysts were ~50% among embryos cryo-stored for ≤90-days, but always <50% for embryos cryo-stored for longer periods, p=0.02). The miscarriage-rate (overall 14%, ranging 7-18%) was not associated with cryo-storage duration already from univariate regressions. Also the gestational (overall 6%, ranging 0-8%) and perinatal issues rates (overall 3%, ranging 0-5%) were not associated with cryo-storage duration already from the univariate regressions. Neither the gestational age nor the birthweight showed significant associations with cryo-storage duration, as confirmed by linear regressions. In fact the rate of newborns whose weight was normal-for-gestational-age was similar across all cryo-storage duration groups (overall 81%, ranging 80-83%).
Limitations, reasons for caution: The prevalence of first transfers decreases from ≥95% for procedures conducted ≤90-days from vitrification to 71%, 39%, 22% and 4% for procedures conducted between 91-180, 181-360, 361-720 and >720-days, respectively. However, also the sequential number of transfer was not associated with the LBR when adjusted for blastocyst-quality and full-blastulation day.
Wider implications of the findings: Cryo-storage by vitrification is confirmed safe in the hands of experienced operators, and its duration does not impact any outcome. This information is valuable for freeze-all cycles, but also for women cryo-preserving surplus embryos for second pregnancies; in this regard, 6.8% of the patients in this study delivered ≥2 LBs.
Trial registration number: not applicable

P-784 Neonatal follow-up of babies born derived from mono-pronuclear zygotes

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Study question: Are the neonatal outcomes normal of babies derived from the transfer of blastocysts derived from mono-pronuclear(1PN) zygotes?
Summary answer: There was no effect on growth or increase in congenital anomalies up to 18-months in babies of 1PN-derived births.
What is known already: 1PN zygotes are observed in ART, albeit at a low rate. We have previously reported that 80.7% of 1PN zygotes derived from IVF or ICSI had a biparental chromosome using Live Cell imaging techniques, and some of these developed to the blastocyst stage (Tokoro *et al*. ASRM 2013). Furthermore, we have reported that these blastocysts can result in a viable pregnancy and healthy live birth (Tsuji *et al*. ASRM2020). However, there is some uncertainty about the developmental mechanism of 1PN zygotes, and there is no clear consensus on their clinical utility.
Study design, size, duration: This was a retrospective study which included 55 cases where there was a live birth after single embryo transfer of a blastocyst derived from 1PN zygote. The incidence of birth defects, birth weight was recorded as well as a physical development survey of 25 children who responded to the 18-months follow-up survey. The time period was 72 months (January 2013 to December 2018).
Participants/materials, setting, methods: Patients seeking fertility treatment at an established private IVF clinic. We compared the birth weight, birth after18-months height and weight of children born to 1PN zygotes with data from a control, 2PN group. Statistical significance was determined using the t-test (level of P<0.05).
Main results and the role of chance: The incidence of birth defects in 1PN embryo-derived infants was 1.8% (1/55). The average birth weight of boys in the 1PN group was 3105.6+/-360.3g, which was not significantly different from 3041.0+/-443.3 g in the 2PN group. In girls, the average birth weight was 3085.7+/-454.9 g in the 1PN group, which was not significantly different from the 2PN group (2938.9+/-311.5 g). The average height at 18-months, was 81.6+/-2.5 cm vs 80.5+/-3.4 cm for boys; 79.0+/-1.8 cm vs 79.0+/-3.4 cm for girls in the 1PN and 2PN groups, respectively. The average body weights of the 1PN and 2PN groups were 11.1+/-1.1 kg vs 10.7+/-1.1 kg for boys; 9.7+/-0.9 kg vs 10.1+/-1.0 kg for girls, respectively. There was no significant difference in average height and weight up-to the 18-months follow-up survey.
Limitations, reasons for caution: The incidence of 1PN derived births is low and the study was limited to cases of single blastocyst embryo transfer.
Wider implications of the findings: The incidence of congenital anomalies in Japan was around 1.7 to 2%, and the incidence was similar in the 1PN. There was no difference in the birth weight and 18-months follow-up survey of the 1PN compared with the 2PN. We have demonstrated that there is clinical utility of 1PN embryo.
Trial registration number: not applicable

P-785 Double versus sequential single blastocyst transfer in freeze all cycles?

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Study question: Is double blastocysts transfer (DET) better than sequential single blastocyst transfer (seq-SET) in freeze all cycles?
Summary answer: Sequential single blastocyst transfer provides a higher live birth rate (LBR) per cycle initiated and eliminates multiple births in freeze-all cycles.
What is known already: Improvements in cryopreservation technology helped freeze-all strategy gain much popularity. The new debate is whether guidance for single embryo transfer should also be applied to frozen-thawed embryo transfers in freeze-all cycles.

Study design, size, duration: We performed a retrospective cohort analysis of 860 women in whom the entire cohort of embryos frozen at the blastocyst stage for various indications. All women aged 19-43 years, who had at least two blastocysts frozen and subsequently thawed and transferred were included. Preimplantation genetic testing cycles were excluded.The study period ranged from January 2016 to May 2019.
Participants/materials, setting, methods: Data regarding female age, number of embryos transferred, multiple pregnancy and live birth rates (LBR) were extracted from the electronic database. Women were categorized based on their age and the mode of embryo transfer. Primary outcome was live birth rate LBR per cycle initiated. Secondary outcomes were LBR per embryo transfer and multiple birth rate. Groups were compared using Fisher's test, generalized estimating equation model and logistic regression analysis to adjust for confounding factors.
Main results and the role of chance: The study group comprised of 666 women (371 Seq-SET and 295 DET) who underwent 837 embryo transfer cycles. Second embryo transfer was affected in 46.1% of women in the Seq-SET group. Age, indication for freeze-all, and mode of transfer were related with the LBR. For women ≤ 35 (n=370), LBRs per embryo transfer were similar in single and double embryo transfers (53.9% versus 64.2% respectively, p=0.006, aOR=0.65, 95% CI:0.41-1.01). However, LBR per cycle initiated was significantly higher in Seq-SET group (78.9% versus 64.2% respectively, p=0.004, aHR=2.09, 95% CI:1.28-3.41). While only one monochorionic twin delivery was observed with Seq-SET (0.5 %), 19 out of 70 (27.1%) live births after DET were twins. For women >35 of age (n=296) the likelihood of a live birth per embryo transfer was lower in single compared to double embryo transfers (33.2% versus 46.2%, respectively, p=0.012, aOR=0.58 95% CI:0.38-0.88). Although LBR per cycle initiated was higher in Seq-SET (58.2%) than DET (46.2%), the difference did not reach statistical significance (p=0.054, aHR=1.62, 95% CI:1.00-2.60). While no twin delivery was observed with Seq-SET, 8 out of 86 (9.3%) live births with DET were twins.
Limitations, reasons for caution: This was a retrospective study with small sample size performed at a single fertility center, which may limit the generalizability of our findings. Cost-efficiency was not studied.
Wider implications of the findings: Seq-SET is associated with a comparable or higher likelihood of live birth per cycle initiated and a very low risk of twins when compared to DET. However, half of SET cases had to undergo two transfer cycles.
Trial registration number: NA

P-786 Virtual continual professional education programs in ART in time of SARS-CoV-2: do they deliver?

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Study question: Can virtual training deliver effective professional education to ART professionals?
Summary answer: Virtual continual professional education programs are an excellent learning platform for ART professionals. The web-based Educational Library is a very useful global scientific resource.
What is known already: Retention levels are the highest when theoretical knowledge sharing is combined with practical hands-on training in a face-to-face training center set up. This is especially the case for training in Assisted Reproductive Techniques, where success depends in part on the ART professional's skills. Due to the global SARS-CoV-2 pandemic in 2020, hands-on training programs were forced to close, and new educational web-based activities tools like streaming of webinars and journal clubs were developed.
Study design, size, duration: The effectiveness of the Global Education and Webinar Series organised by CooperSurgical (including webinars and journal clubs) streamed in 2020, was evaluated retrospectively by analysing the following: 1) the live attendance rates; 2) viewing rates in the Webinar Series Library; 3) outcomes of the feedback questionnaire focusing on the level of the webinar content, relevance to day-to-day clinical and laboratory work, gaining new knowledge, and pace of the webinar.
Participants/materials, setting, methods: In 2020, 65 webinars and 8 journal clubs were streamed at different timepoints to accommodate a global professional ART audience. The target audience included embryologists, lab technicians, IVF clinicians, counsellors, and scientists. Topics were IVF lab and clinic-based, theoretical but also practical. Lectures were prepared with an evidence-based approach and submitted for scientific review. Post live attendance, viewers were invited to fill in a questionnaire; they obtained a certificate of attendance.
Main results and the role of chance: In 2020, 16,839 viewers attended the 65 live webinars and 8 journal clubs. Live attendance rates dropped by 75% in May, when IVF clinics were re-opening after the first wave of SARS-CoV-2.

On 08.01.2021, a total of 23,258 library viewings were recorded. Library viewings increased significantly after the re-opening of the clinics.

Viewers were located in 129 countries; India, Thailand, and Spain had the highest viewing of all the countries (> 1500 viewings per country). Multiple viewers attended between 10 to 26 of the virtual activities.

The feedback analysis showed that 96% of the viewers found the webinars to be relevant to their day-to-day work; 92% gained knowledge as a result of the webinar; 94% of the viewers found the level appropriate and 91% felt that the pace of the presentations was just right.

These outcomes demonstrate that the need for continual professional education programs in ART in time of SARS-CoV-2 is clearly present globally. Our virtual Global Education and Webinar Series could deliver evidence-based knowledge to viewers globally and assist them in gaining knowledge – even in a distance learning setting. The Library is an excellent resource tool for ART professionals to gain knowledge at their own pace.
Limitations, reasons for caution: Not all ART professionals have access to high-quality internet facilities. Not all the viewers completed the questionnaire
Wider implications of the findings: Web-based virtual activities can be an excellent tool for knowledge sharing. These outcomes will be used to further develop our virtual educational training program.
Trial registration number: Not applicable

P-787 Impact of delaying ART to promote weight loss: a large multicentre study accounting for the combined effect of female/male age and body mass index (BMI)

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Study question: Is postponing the start of ART (to promote a reduction in female BMI) beneficial for cumulative live birth rates (CLBR) when accounting for the female/male ageing this delay will cause?
Summary answer: Postponing ART treatment in one year to promote female weight loss could be detrimental in women of advanced maternal age (AMA, >35 years-old).
What is known already: Overweight/obese couples are frequently encouraged to lose weight prior to infertility treatment to enhance ART outcomes. However, a meaningful weight loss is often difficult to achieve for these couples, frequently taking at least one year to accomplish. Given that both female and male ageing are also important for ART success, we were interested in understanding the combined impact on CLBR of BMI reduction and ageing following a one-year delay.
Study design, size, duration: A retrospective study including patients performing their first ART cycle using autologous gametes between 2013-2018 in one of 39 participating ART centres. Only GnRH antagonist cycles were included (n=14260). CLBR was the primary outcome. Secondary outcomes included time-to-pregnancy, birthweight and gestational age.
Participants/materials, setting, methods: Patients were subdivided according to female BMI (Kg/m2) in either underweight (<18.5), normal-weight (18.5-24.9), overweight (BMI 25.0-29.9 kg/m2) and obese (≥30 kg/m2). Meaningful and extreme weight loss were defined as a reduction from obesity to either overweight or normal-weight, respectively. We performed multivariable regression analysis to account for potential confounding.

Main results and the role of chance: Overweight (36.8%) and obese (33.0%) women had significantly lower CLBR when compared to the underweight (42.6%) and normal-weight (41.4%). When assessing the confounder-adjusted net-effect of male/female age and BMI, the predicted benefit of promoting a meaningful BMI reduction was lower than the estimated hindrance due to male/female ageing as soon as women reached AMA (n=8365, 58.6%). This absence of benefit was especially important in women >38 years-old, in which even extreme weight-loss did not compensate for the age-related reduction in CLBR caused by the one-year delay. Moreover, male weight-loss failed to provide any additional benefit when accounted for in the regression models. Finally, obesity was also associated with a modest but statistically significant one-month delay in time-to-pregnancy and a 96.1 g (95% confidence interval: 39.9-152.4) increase in birth weight. The diagram of predicted outcomes presented in this study may serve as a useful tool to counsel patients before treatment, namely when recommending treatment postponement to promote short-term (i.e. 3-6 months) or long-term (i.e. 1 year) weight loss.
Limitations, reasons for caution: Caution is recommended when extrapolating these results into everyday practice owing to the retrospective nature of the study and the fact that only GnRH antagonist cycles were included.
Wider implications of the findings: Patients are frequently confronted with the dilemma to either postpone treatment (and promote weight loss) or start treatment immediately (to avoid further ageing). Our results seem to show that women in AMA may have hindered CLBR if recommended to delay treatment even if the desired weight loss is ultimately achieved.
Trial registration number: Not applicable

P-788 Health outcomes at birth, 12 and 24 months of 747 children conceived after Preimplantation Genetic Testing: a single centre experience

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Study question: Does conception by Preimplantation Genetic Testing (PGT-M, PGT-SR) adversely affect health outcomes in children born through this assisted reproductive technique?
Summary answer: No significant difference was noted in the rate of congenital malformations in children born after PGT-M and PGT-SR compared with IVF-ICSI children.
What is known already: It is already known that the risk of congenital anomalies in IVF-ICSI pregnancies is higher when compared with pregnancies conceived naturally.
Study design, size, duration: This is a prospective study on 747 children born between December 1999 and July 2016 after a cycle of PGT-M or PGT-SR (IVF +/- ICSI + embryo biopsy) performed at a single London reproductive centre. PGT-A is not performed in the Centre, so pregnancy outcomes in this group are not relevant. The children were examined at birth, at 12 and 24 months of age and the data collected in three questionnaires.
Participants/materials, setting, methods: 747 PGT-M and PGT-SR children were enrolled in the study. 742/747 were examined at birth, 444/747 at 12 months and 168/747 at 24 months. The assessment consisted of three separate questionnaires completed at birth, 12 months and two years of age. The first questionnaire focused on the detection of congenital anomalies in newborn babies. The questionnaire at follow up recorded growth data and examination of the baby's health and development.
Main results and the role of chance: We found no evidence that PGT-M and PGT-SR increased the risk of an adverse perinatal outcome when compared with children born after IVF-ICSI. The overall malformation rate in our group of live born after PGT-M and PGT-SR was 3.9% and of major malformations was 2%. These values are comparable with literature data on malformation risk in children born after IVF-ICSI. In terms of misdiagnosis, we had one misdiagnosis of SMA type 1 in 658 pregnancies obtained. This was very early on in the centre's experience of offering PGT-M. Follow-up visits in our cohort allowed us to evaluate their development. Unfortunately, the low participation rate at 24 months (23%) significantly reduced the size of our cohort. We observed a cumulative value of 10% at 24 months of babies with developmental delay which is comparable with the value of 10% given by the WHO, but is twice the incidence Global Research on Developmental Disabilities Collaborators described in the UK in 2016 (4.6%). To our knowledge, no large studies have assessed the risk of developmental delay in children born after PGT. We cannot draw conclusions on this from our small cohort at 24 months and recommend further studies.
Limitations, reasons for caution: Although our sample is one of the largest reported, it is too small to generalise results due to the heterogeneity of the conditions for which PGT was being offered and the rarity of these conditions. There were multiple confounding factors including couple's fertility background, varying fertility treatments and embryological techniques.
Wider implications of the findings: Our results support published literature highlighting the safety of PGT-M and PGT-SR techniques. We followed up at birth, 12 months and 24 months a large cohort of children, in one of the largest datasets published so far.
Trial registration number: not applicable

P-789 Obstetric and perinatal outcomes in poor ovarian responders and normal responders flowing fresh embryo transfer: a prospective, monocentric, observational study

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Study question: Whether women pregnant after a poor response in IVF have obstetric and perinatal complications more frequently than women with pregnancies after a normal response in IVF?
Summary answer: There were no statistically significant differences in obstetric and perinatal complications rate between poor responders and normal responders in Chinese women.
What is known already: Poor ovarian response usually indicates a reduction in follicular response, resulting in a reduced number of retrieved oocytes. Patients are less likely to conceive and have a higher risk of cycle cancellation and low clinical pregnancy rate. Whether poor ovarian response is associated with obstetric and perinatal complications is however debated.
Study design, size, duration: Design: Prospective, monocentric, observational study.
Size: 1664 women with poor ovarian response and 1061 women with normal ovarian response
duration : July 1, 2017 to Aug 15, 2019.Participants/materials, setting, methods: 1664 women with poor ovarian response and 1061 women with normal ovarian response undergoing IVF or ICSI were enrolled in this study. The primary outcome was obstetric and perinatal complications rate.
Main results and the role of chance: 1664 women with POR and 1061 women with NOR were enrolled in this study.Poor and normal responders did not have significantly different incidences in obstetric and perinatal complications(25.42% vs 25.45%), nor were there a significant difference in preeclampsia, gestational diabetes mellitus, postpartum hemorrhage or abruptio placentae. But POR group have a lower frequency of twin pregnancies(8.47% vs 28.66%,P<0.01),low birth weight(5.08% vs 14.23%,P<0.01)and prematurity (9.32% vs 17.03%,P<0.01).
Limitations, reasons for caution: Despite its size, an observational study such as this has a number of inherent limitations, and the best way to confirm its findings will be to compare obstetric and perinatal outcomes in different subgroup of pregnancies following ART in an adequately powered randomized, controlled trial.

Wider implications of the findings: This prospective, monocentric, observational study suggests that women with poor ovarian response did not have higher perinatal complication rate than women with normal ovarian response. Oocyte quality and quantity may not affect the rate of perinatal complications.
Trial registration number: not applicable

P-790 Effects of COVID-19 quarantine period on Fertility Treatment and IVF Clinic management

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Study question: What are the effects of the initial COVID-19 response on the management of fertility clinics and clinical practice around the world?
Summary answer: In the COVID-19 outbreak, the large majority of fertility clinics worldwide suspended fertility treatments. In cycles that continued, there was a shift to frozen embryo-transfer.
What is known already: After the initial months of 2020 showed a rapid spread of the new Coronavirus SARS-CoV-2, the World Health Organisation declared a global pandemic on 11 March 2020. Occupation of health care facilities with acutely sick patients and the need to reduce infection transmission led to a reduction in capacity to perform elective medical procedures. Little was known on the global impact of COVID-19 on fertility care. With the implication of 'lockdowns' in different countries around the world to stop the spread of the virus, the question was posed on how fertility clinics and treatments would proceed moving forward.
Study design, size, duration: We surveyed fertility clinics with an online questionnaire developed through the platform RedCap (HELIX). The questionnaire contained 33 questions focused on the differences of country responses to different body guidelines including American Society for Reproductive Medicine (ASRM) and European Society of Human Reproduction and Embryology (ESHRE). Fertility clinic associates were contacted through the use of a known contact list comprising scientific directors, medical directors and lab managers.
Participants/materials, setting, methods: Study participants were individuals associated with fertility clinics around the world with at least one representative from each country. The questionnaire was active from 13th October 2020 until 21st January 2021. The time frame was specific to the country's response to their first lockdown. The survey was approved by Monash Health Human Research Ethics Committee(#65223). All survey answers were anonymous with only the countries' name as a reference for analysis.
Main results and the role of chance: There were 34 individual country responses,Asia(11), Europe(10), Africa(3), North America(3), Oceania(2) and South America(5). Of the 34 countries, 7 countries did not experience a complete stop of all procedures. Most countries (18) followed their government body recommendations. One country followed local recommendations, 3 followed local and international recommendations, 3 countries changed by clinic initiative and 7 countries did not specify. ASRM and ESHRE were the two most common guidelines mentioned. IVF/ICSI treatment had delays in 28 countries ranging from 14 (Scotland) to 160 (Egypt) days. FETs were delayed in 29 countries ranging from 15 (Pakistan) to 228 (Scotland) days. Couples undergoing timed intercourse experienced the least delay in treatment (13 countries). AI/OI (artificial insemination/ovulation induction) patients were delayed treatment in 25 countries, fertility consultations were delayed in 20 countries. During the quarantine period, the amount of freeze-all cycles increased in 16 countries with the ratio of IVF-ICSI remaining constant pre and post lockdown. Patients were reported to undergo a SARS-CoV-2 test in 17 countries. 11 countries reported having a procedure in place for patients whom tested positive, 6 countries reported no procedure in place for positive patients. Additional support counselling was offered for patients during the pandemic in 22 countries.
Limitations, reasons for caution: Our survey does only represent a minute sample of countries. As only one representative from each country was used, the results obtained are specific to the individual's anonymous clinic. However, the questionnaire includes questions that specifies if the clinic was performing outside the country's scientific society recommendations.
Wider implications of the findings: During the COVID-19 pandemic most fertility services were suspended, providing insight to the implications of a shutdown and whether a protocol for scenarios of this nature could benefit outcomes for future events. A protocol that allows continuation of care,including telehealth and guidelines for prioritizing couples who need care most urgently.
Trial registration number: N/A

P-791 Comparison of the live birth rate after single embryo transfer in fresh versus frozen cycle: no evidence to support routine freeze-all strategy

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Study question: Is the treatment outcome compromised after superovulation for fresh IVF/ICSI in comparison to frozen cycle with spontaneous ovulation and luteal support with progesterone?
Summary answer: Live birth rate (LBR) is dependent on embryo quality both in the fresh and frozen cycles with no sign of harmful effect of the superovulation.
What is known already: Freeze-all strategy has been advocated in recent years based on the assumption that luteal phase after superovulation is not optimal for embryo implantation. The effects of variable hormonal treatments, given in association with ART, on the endometrium, are still largely unknown. Therefore, more data is needed in order to optimize the treatment policies.
Study design, size, duration: This is an observational retrospective single-center cohort study. Data were collected from Oulu University Hospital's ART-database including a total of 5647 single embryo transfer cycles from years 1995-2020. Patients stimulated with long agonist protocol for IVF/ICSI and day 2-3 transfer were included. Frozen embryo transfer was performed in a natural cycle with an ovulation test used for timing of transfer. Luteal support with progesterone was given for two weeks in all cycles.
Participants/materials, setting, methods: There were 3053 IVF/ICSI fresh cycles (2237 top and 816 N-top) and 2594 frozen cycles (806 top and 1788 N-top). The main outcome measure was LBR compared between fresh and frozen cycles when either a top or a N-top embryo was transferred. As a secondary outcome, clinical pregnancy rate was investigated. Data on the age and body mass index (BMI) of the patients was available. Student's T-test was used to compare continuous variables.
Main results and the role of chance: The groups did not differ regarding the age and BMI of the patients. After the transfer of a top quality embryo the clinical pregnancy rate (35.4 vs. 30.8 %; $p<0.05$) and LBR (29.4 vs. 25.5%; p not significant) was slightly higher in the fresh cycle. After the transfer of a N-top quality embryo the clinical pregnancy rate (19.9 vs. 19.4 %) and LBR (15.9 vs. 15.4 %) were similar both in the fresh and frozen cycles.
Limitations, reasons for caution: This study only represents cleavage stage embryo transfers, and all FETs were performed in a natural cycle. In a retrospective study there may also be residual confounding that could not be excluded.
Wider implications of the findings: This study provides further evidence that treatment outcome regarding LBR is not affected by superovulation therapy. Hence, the use of freeze-all strategy is warranted only in cases with a risk of ovarian hyperstimulation syndrome.
Trial registration number: Not applicable

P-792 The introduction of an embryo morphokinetics annotation quality assurance scheme across ten fertility clinics including 59 participants

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Study question: Can a group-wide quality assurance scheme be developed to effectively determine inter-operator agreement for morphokinetic parameters of interest.
Summary answer: Very strong agreement was found between all operators except for one, therefore this scheme effectively identified areas of improvement in inter-operator annotations.
What is known already: Where fertility clinics use embryo morphokinetics to determine viability potential, quality assurance of annotations is essential. Embryo selection algorithms rely on the manual determination of certain morphokinetic parameters. Variations in these parameters can lead to differences in the algorithm score attributed to an embryo thus potentially affecting its fate. It is vital that all embryologists involved in embryo annotation and selection are consistent with their annotation approach through regular quality assurance mechanisms.
Study design, size, duration: Each participant was required to annotate the same three embryos for morphokinetic parameters of interest, including tPB2, tPNf, t2 to t5, t8, tM, tSB, tB. Participants were also required to grade embryos at 68 hours post insemination (hpi), 112hpi and to assess additional parameters used for embryo selection or future investigations, such as the extent of morula compaction. The aim of this scheme is to release new distribution each quarter to ensure regular participation.
Participants/materials, setting, methods: All embryologists responsible for embryo annotation in a single, UK fertility group were enrolled onto the scheme. A total of 59 participants from 10 fertility clinics in the UK were included. Inter-operator agreement was assessed using two-way, mixed intraclass correlation coefficient (ICC) for consistency. Five categories of agreement were determined based on ICC score; very weak (0-0.2), weak (0.21-0.4), moderate (0.41-0.6), strong (0.61-0.8) and very strong (0.81-1.0).
Main results and the role of chance: Very strong agreement (0.81-1.0) was observed between all operators for all parameters assessed except for one operator who showed a weak agreement (0.21-0.4) with all other operators. Descriptive statistics revealed standard deviations (SD) ranging from 0.34 (t3) to 3.43 (t5). For each parameter the SD across the three assessed embryos ranged from 0.34-3.43; tPB2 (0.11-0.98), tPNf (2.06-4.40), t2 (0.22-0.80), t3 (0.16-0.70), t4 (0.39-0.65). t5 (2.40-5.44), t8 (0.33-2.72), tM (1.00-2.72), tSB (1.08-2.67), tB (1.12-1.81). These results indicate a high concordance with less subjective annotations such as the cell stage divisions and more variability with the subjective annotations such as the blastulation parameters. The concordance with less well practiced or understood annotations, such as extent of morula compaction, planar or tetrahedral orientation at the four cell stage as well as angle of extrusion of second polar body in relation to the first polar body, was poorer as indicated using descriptive statistics. This highlighted the need for experience in performing these annotations before drawing conclusions regarding their predictive nature in relation to an embryo's viability.
Limitations, reasons for caution: The variability between more subjective parameters would be expected to be higher than others. The participation in these schemes can create false environments which do not reflect how an embryologist would usually score; they may spend longer on some decisions given the nature of the scheme.
Wider implications of the findings: Quality assurance of morphokinetic annotations across clinics utilising standardised selection models is crucial. Robust annotation policies and education programmes are essential in achieving consistent results between operators. Quality assurance schemes can identify individuals who lack consistency overall and can identify reliably annotated parameters to inform inclusion in embryo selection algorithms.
Trial registration number: Not applicable

P-793 Validation of French in vitro fertilization (IVF) guideline during Covid-19 pandemic by the research of Sars-Cov-2 RNA in the follicular fluid (FF) after egg retrieval

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Study question: Is it possible to find viral Sars-Cov-2 RNA in FF of women undergoing treatment during Covid-19 pandemic that may compromise gamete and embryo safety?
Summary answer: No viral RNA was detected in tested FF of women undergoing IVF in compliance with recommendations. This was reassuring and supported good medical practice.
What is known already: Risks due to SARS-CoV-2 during IVF remain difficult to assess despite the screening recommended by French health authorities based on a symptom questionnaire of the couple (systematic testing by RT-PCR for the virus before egg retrieval (ER) is not mandatory). In this context, this is a real challenge for IVF laboratory to guarantee procedure, patients, gametes and embryos safety. Most studies have reported the absence of virus in sperm. No data are available for FF and only one study looked for the presence of the virus in oocytes of Covid-affected patients (Barragan M *et al*, 2020).
Study design, size, duration: Between June 17 and September 24, 2020, FF of consenting women were prospectively collected and symptom questionnaire recorded. During this period, women undergoing IVF in our center did not benefit from systematic PCR testing for the virus within 72 hours prior to ER through our health authorities' recommendations. All collected FF were retrospectively tested to research viral RNA by RT-PCR and patients were recalled to answer an epidemiological follow-up questionnaire.
Participants/materials, setting, methods: For all couples, symptom questionnaires were prospectively recorded and verified at each step of IVF procedure. For all consenting women, a sample of 1 ml of FF was collected the day of ER and stored at -80°C. After thawing, a Sars-Cov2 multiplex RT-PCR using CFX96 (Biorad*) was performed, after RNA extraction using Nimbus (Seegene*). A comprehensive epidemiological evaluation was made afterwards by phone interview and data were recorded and analyzed.
Main results and the role of chance: A total of 183 women was included out of the 214 treated during this period (85.5%). Retrospective epidemiological evaluation showed that 8 patients contracted Covid more than 2 months before the ER, 6 more than 2 months after and only one patient 1 month after ER (diagnosis based on pathognomonic signs as agueusia and anosmia or/and positive PCR). We observed a prevalence of symptomatic Covid forms in our IVF population of 8.2% during a 6-month period surrounding their IVF cycle. Moreover, until the introduction of systematic testing by RT-PCR for the virus before ER since the end of September 2020, 3 patients have been cancelled out of the 403 planned for positive PCR despite a negative questionnaire, which represents a prevalence of asymptomatic forms on the day of the ER at 0.7%. All the 183 FF tested did not reveal any viral RNA detection, which was reassuring concerning our medical practice and patient compliance and transparency. The absence of detected viral RNA may be due to several reasons: 1) women were not infected the day of ER 2) women had an asymptomatic form of the disease with low viral load 3) FF is not a virus reservoir.
Limitations, reasons for caution: Not all patients were included (85.5%). Post-diagnosis stays uncertain because PCR tests at the beginning of the epidemic were not mandatory and hardly available.
Wider implications of the findings: The absence of viral RNA in FF of women only screened through a symptom questionnaire is reassuring concerning the safety of IVF during Covid pandemic.
Trial registration number: not applicable

P-794 Prevalence of positivity for SARS-CoV-2 RNA in follicular fluid in infertile patients

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Study question: Is Sars-Cov-2 present in the follicular fluid of infertile patients?
Summary answer: In the experience of the Infertility and IVF Unit, University Of Bologna, Italy, RNA of SARS-Cov-2 was not detected in the follicular fluid.

What is known already: Data on the risk of virus presence in reproductive cells and transmissibility in IVF procedures are very limited. In literature only one study reports the detection of SARS-Cov-2 viral RNA in oocytes of PCR positive women. Research of RNA in follicular fluid could be a marker able to indicate whether to continue IVF treatments in the case of swab-positive patients.
Study design, size, duration: Prospective study performed at Infertility and IVF Unit, Sant'Orsola University Hospital, University of Bologna, Italy, from March 2020 to January 2021. 451 IVF cycles were performed on 902 patients. In addition 59 cycles of oocyte cryopreservation were also performed to fertility preservation in oncological patients. In all positive swab patients was analyzed the follicular fluid for RNA virus detection.
Participants/materials, setting, methods: 961 patients underwent telephone triage before going to the IVF Center to identify subjects with suspected or confirmed infection. Body temperature was measured on all patients before entering the IVF Center.

All patients were subjected to real-time analysis (RT PCR) of pharyngeal swab samples 48 hours before transvaginal ultrasound-guided oocyte retrieval.

In case of positive swab, PCR was performed on follicular fluid.
Main results and the role of chance: In our population of infertile patients the incidence of SARS COV-2 infection positivity was 0.4% (4/961).

No IVF treatments were suspended. The oocytes of the 4 women with positive swab were cryopreserved using closed devices stored in a special dedicated cryogenic container. No viral RNA was detected in the follicular fluid.
Limitations, reasons for caution: there are no limitations to the study.
Wider implications of the findings: The absence of SARS-COV-2 RNA in the follicular fluid is a reassuring result in the storage and future use of oocytes.
Trial registration number: not applicable

P-795 Assessment of the risk of contamination of semen, follicular and vaginal fluids with SARS-CoV-2 virus in patients undergoing ART

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Study question: Is SARS-CoV-2 detected by RT-PCR in the reproductive materials and follicular fluid of asymptomatic patients undergoing fertility treatments?
Summary answer: No SARS-CoV-2 mRNA was detected in sperm, vaginal and follicular fluids samples of asymptomatic patients, irrespective of the nasopharyngeal swab or COVID-19 questionnaire results. What is known already: The COVID-19 pandemic had a huge impact on health care including on fertility clinics. While activities were interrupted during the first wave, ART cycles are currently performed but uncertainties remain regarding the presence of the virus in reproductive materials. The SARS-CoV-2 receptors were detected in reproductive organs but only few studies with limited number of cases reported the presence of SARS-CoV-2mRNA in semen of symptomatic patients. In women, the risk of SARS-CoV-2 contamination in follicular and vaginal fluids remains uncertain. Thus the risk of sexual transmission and the safety of the IVF laboratory procedures are unclear.
Study design, size, duration: This COVART study is an observational cohort prospective trial conducted at a Belgian academic hospital. Between September 2020 and January 2021, 208 asymptomatic adults patients (men and women) undergoing ART treatments (sperm analysis, IUI, ICSI/ICF cycles, oocyte cryopreservation) were included in the trial after informed consent. All patients followed standard procedures to evaluate COVID-19 risk (nasopharyngeal swab during ovarian stimulation and COVID-19 risk questionnaire). Participants were divided into two groups: COVID-19 positive and negative/unknown groups. Participants/materials, setting, methods: Swabs on the residual reproductive materials were done and stored in viral transport medium at 4°C until processing. After addition of an internal control in each sample and virus inactivation with Trizol, mRNA was extracted using phenol-chloroform method. Quantitative RT-PCR was performed in duplicate following a previously validated protocol (45 cycles, Roche Light Cycler 480). Negative/positive controls were used to validate each run. The test was considered as positive when CT < 40.
Main results and the role of chance: A total of 399 samples (126 semen, 162 vaginal fluid, 111 follicular fluid samples) of reproductive residual materials from 208 participants were collected during the peak of the second wave of COVID-19 pandemic, when Belgium was considered as a red zone with a viral Rt of 1.516 and a 14-day COVID-19 cases notification rate above 630 per 100000. Although the policy of the fertility clinic was to cancel all cycles of patients with a positive nasopharyngeal swab test except if specific medical raisons to continue the cycle, 14 samples from 9 non-cancelled patients diagnosed with COVID-19 before or just after the samples collection were analyzed (4 sperm, 5 follicular fluid and 5 vaginal secretion samples). For the 199 remaining patients, the COVID-19 status was negative or unknown. None of the samples were considered as positive after quantitative RT-PCR analysis.
Limitations, reasons for caution: All the patients were asymptomatic at the time of the samples collection and the large majority was negatively diagnosed for COVID-19 during the ART cycle. The results should be confirmed by including a larger cohort of positive patients. Data on the impact on ART outcomes will be evaluated.
Wider implications of the findings: We showed that contaminations of sperm, follicular and vaginal fluids with SARS-CoV-2 are unlikely in asymptomatic patients, even when diagnosed positive, confirming the poor risk of sexual transmission. Moreover, no additional safety measures seems to be implemented in the IVF laboratory to ensure the safety of the staff.
Trial registration number: P2020/414

POSTER VIEWING
STEM CELLS

P-796 Trial of Autologous Marrow derived Stem Cell Ovarian Transplantation (TAMSCOT) in young infertile women with diminished ovarian reserve for ovarian rejuvenation – HOPE still persists

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Study question: Does autologous bone marrow derived stem cell (BMDSC) ovarian transplantation optimize ovarian reserve parameters in young infertile women with diminished ovarian reserve (DOR) ?
Summary answer: The autologous stem cell ovarian transplantation (ASCOT) improves AFC and AMH by facilitating the recruitment of existing dormant follicles in young women with DOR.
What is known already: Oocyte donation is the practical therapeutic option when patients with premature ovarian ageing desire pregnancy. It involves significant psychological burden in terms of not able to have their own biological child. ASCOT has opened new doors in poor responders and premature ovarian insufficiency through its beneficial effects on ovarian reserve and IVF outcomes. However recent studies have shown contradictory results in terms of its efficacy. No prior study has been contemplated in DOR group
Study design, size, duration: An open label non randomized controlled trial was conducted at Division of Reproductive Medicine in collaboration with stem cell facility at tertiary care institute. Forty two infertile women less than 35 years age with DOR (AFC<5, AMH<1.2ng/ml and /or high FSH>8IU/l) were enrolled in the study during a period from January 2020 to December 2020. 20 women who did not opt for the intervention were treated as control group whereas 22 women received the intervention.

Participants/materials, setting, methods: Baseline hormonal profile (Day 2 FSH, estradiol, AMH and AFC) was done in all patients. Women with abnormal uterine cavity, endometriosis, prior ovarian surgery, abnormal karyotype were excluded. Bone marrow aspiration followed by mesenchymal stem cells isolation was performed. The stem cells were transplanted in both the ovaries through transvaginal route on the same day. Follow up visits were planned at one and six months to assess ovarian reserve parameters.
Main results and the role of chance: The mean age, BMI and duration of infertility were comparable between the control and study group (29.5±3.34vs29.36±2.95years, 21.51±1.40vs21.87±1.93kg/m2, 6.9±1.94vs7.04±3.67 years) . The positive response in terms of improved AMH and AFC was seen in 68% (15/22) patients. The mean number of stem cells injected in these women were 77.71±25.33 million. At first follow up, there was no significant difference between mean FSH, estradiol levels and mean right and left ovarian volume (9.23±3.95 vs 9.02±3.92mIU/l, 61.46±29.25 vs 68.12±62.52 pg/ml, 2.82±2.18 vs 2.44±1.25 cc, 2.02±1.54 vs 2.72±1.06 cc, p<0.05). There was significant increase in AMH and AFC values as compared to baseline (0.79±0.43 vs 1.26±0.82ng/ml, p=0.03; 3.47±1.30 vs 6.40±2.23, p<0.001). At second follow up visit, the significant increase in ovarian reserve persisted for AMH and AFC (0.79±0.43 vs 1.22±0.76 ng/ml, p=0.02; 3.47±1.30 vs 6.93±1.71,p<0.001). There was no significant difference between serum FSH , Estradiol and ovarian volume. None of the patients developed any complication and the improvement in AFC and AMH persisted during 10 month follow up period.
Limitations, reasons for caution: The limitation of present study is small sample size and non randomization. However, time period for which positive effect lasts has not been documented in earlier studies. This study is currently being endeavored, and women with improved ovarian reserve are followed up for any spontaneous conception or following assisted reproduction.
Wider implications of the findings: The present study demonstrates beneficial role of stem cells in improving ovarian reserve parameters in women with DOR with no acquired cause. If supported by future randomized clinical studies, it could represent a paradigm shift for fertility treatment in these women providing an opportunity to have their own biological child.
Trial registration number: CTRI/2020/01/022726

P-797 A novel method for establishing human embryonic stem cells independent of feeder cells

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Study question: Is there a efficient establishing method of human embryonic stem cells directly from the human blastocysts independent of feeder cells?
Summary answer: We established a novel method of generating human embryonic stem cells directly from human blastocysts independent of feeder layer cells.
What is known already: Establishing embryonic stem cells lines mainly needed to coculture ICM clumps with feeder cells (like mouse or human fibroblasts) ,this brought in potential heterogeneous pollution.Although there had be some reports about generating human ESCs independent of feeder cells,but the efficiency was low and conditioned medium were unstable and also had the biological contamination.
Study design, size, duration: We used ten day5/6 donated human blastocysts from our reproductive center ,most of them were genetically diseased embryos with abnormal PGT diagnosis.After establishing ESCs procedure , all the cell lines were identified with pluripotency and differentiation potential tests. The success rate of system was calculated and compared with the conventional methods.
Participants/materials, setting, methods: In brief, ICM clumps were separated mechanically by using a micromanipulation system,and then transferred to a 30ul mTESR plus culture media drop pretreated with the geltrex (1:100 dilution) matrix and oxygen concentration was 5%. When cells attached and migrated,we also used laser to destroy the remaining trophoblast cells.About 10 days,the typical ES clone can be mechanically passaged and cells can be cultured in normal oxygen concentrations after passage 2. .
Main results and the role of chance: Using this method we had successfully established nine embryonic stem cell lines from donated human blastocysts ,the success rate was 90% (9/10). Each cell lines had passed the evaluation test of embryonic stem cell. When compared with the conventional feeder cells dependent method,our novol methods not only eliminated the pollution from heterogeneous cells,but also had higher success rate (90% vs 25%).
Limitations, reasons for caution: Due to the scarcity of donated human blastocysts, this experiment was a single-center experiment with small samples.
Wider implications of the findings: We speculated that the batch differences of culture dishes, matrix and culture medium might affect the establish efficiency , and how to carry out a high level of quality control work might be the key factor to keep the system stable.
Trial registration number: basic research

P-798 Fertility preservation in pre-pubertal boys with cancer: A three-dimensional prepubertal testicular organoid for in vitro spermatogonial stem cell propagation and spermatogenesis

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Study question: Can a three-dimensional (3D) prepubertal testicular organoid be formed and provide an *in vitro* microenvironment for spermatogonial stem cells (SSCs) maintenance and future spermatogenesis?
Summary answer: Primary cells extracted from immature testicular tissue (ITT) or SSCs can be grown long-term as 3D organoids, providing the potential for *in vitro* study.
What is known already: Aggressive cancer treatments, such as chemo- or radiotherapy, can leave young prepubertal boys infertile. Such patients are recommended to undergo the cryopreservation of testicular material to protect future fertility. Within the testes, the specialized 3D structure and direct cell-to-cell interactions play a critical role in the proliferation and development of SSCs. Over recent decades, 3D culture systems and organoids have been used to culture cells *in vitro*, however, a system that allows investigations into testicular organogenesis *in vitro*, and its impact on the SSC niche, has yet to be developed.
Study design, size, duration: This study aims to develop a 3D organoid culture system to support the proliferation of SSCs and spermatogenesis. Primary bovine ITT cells and enriched SSCs were isolated and 3D organoids were generated by *in vitro* culture for up to 40 days. Organoid formation was observed after using different foundation cells seeded at different densities and cultured in medium containing gonadotropic supplements.
Participants/materials, setting, methods: Post-thaw bovine ITTs (2 weeks-of-age) were dissociated using two-step enzymatic digestion. Enriched SSCs were selected by Percoll gradients and differential plating. Viability and apoptosis were evaluated by trypan blue staining and TUNEL assays, respectively. SSCs were evaluated immunocytochemically for germ-cell markers (PGP-9.5, PLZF) and Sertoli cell markers (Vimentin, Sox9). Expression levels of SSCs and spermatogenesis-related genes (*Plzf, Gfrα-1, Nanog, Oct4, Stra8, Thy1*) were determined by real-time quantitative polymerase chain reaction (RT-qPCR).
Main results and the role of chance: The viability of digested cells from thawed ITTs was 78.667% ± 2.03. Total testicular cells (<10% SSCs) and enriched SSCs(>50% SSCs) were observed to self-assemble into structurally complex organoids recapitulating the cell type compartmentalization of the testis, in a 3D Matrigel-based culture system with 10% knockout serum replacement (KSR) culture medium, but not with 10% fetal bovine serum(FBS) medium. Testicular organoids were found to exhibit either a grape-like structure and a round-shape structure. Cytoplasmic extensions of spermatogonia/Sertoli cells were in contact with each other within a forming colony. Organoids were formed faster and larger when seeded at a final concentration of 1.5 × 106 cells/ml, compared to 5 × 105 cells/ml and 1.5 × 105 cells/ml. Organoids grew to a diameter of 400 µm within 10 - 15 days and were passaged by mechanical disruption at a ratio of 1:3 every 7 - 10 days. Immunocytochemistry results showed that clusters of PGP9.5 and PLZF-positive cells were present within the organoids. The expression of selected germ cell and spermatogenesis markers in the testicular organoids closely resembled that of primary testicular cells for up to 20 days of culture.
Limitations, reasons for caution: We used calves (2 weeks-of-age) as an animal model to study testicular organoids. This tissue may act differently than human tissues and may not fully represent prepuberty. Furthermore, we only evaluated gene expression levels for selected markers that may not represent the full functional capability of germ cells.

Wider implications of the findings: Testicular organoids, as an *in vitro* bioengineering testicular model, could potentially be used to study testicular tissue development, cellular interactions, endocrinology, and spermatogenesis, in the laboratory but may also be applied for clinical purposes in the future.
Trial registration number: University of Oxford

P-799 Long-term maintenance and meiotic entry of early germ cells in functionalized murine testicular organoids mediated by 3D printed scaffolds and air-medium interphase cultivation

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Study question: Can improved culture conditions advance the functionality of murine testicular organoids (TOs)?
Summary answer: Testicular cells formed spheroidal TOs resembling the functional unit of the testis and supporting meiotic entry of germ cells during long-term culture in printed macropores.
What is known already: Organ cultures at the air-medium interphase have traditionally been used for in-vitro spermatogenesis (IVS) in rodents because they best preserve the testicular architecture, which is pivotal in achieving IVS. However, organ cultures do not offer the ability to access and manipulate single cells, making it an inefficient model for mechanistic studies. Culturing testicular cell suspensions into organoids offer these features. Previously, testicular organoids in immersion culture resulted in testicular architecture, but only supported short-term survival of germ cells. Moreover, millimeter-sized organoids show signs of degeneration due to insufficient nutrient and oxygen supply.
Study design, size, duration: First, we focused on recreating the testicular architecture at air-medium interphase and determined whether higher cell densities could improve our previously developed 3D printed culture model during long-term culture using different mouse strains. Afterwards, the focus was put on improving TO morphology by adapting the scaffold design. Moreover, to expand the potential of TOs, the possibility to cultivate chimeric mixtures of testicular cells and germ line stem cells expressing a reporter transgene (EGFP) was assessed.
Participants/materials, setting, methods: Prepubertal testicular cells from C57BL/6J (n=5) or CBAB6F1 (n=3) mice were cultured in the macropores of 3D printed squared 1-layered scaffolds (1LSs) composed of Cellink-RGD (8x104 cells/mm²). Next, 1LS was modified with an additional layer of alginate (2LS) to culture a chimeric mixture of testicular cells of prepubertal C57BL/6J mice and EGFP-expressing germline stem cells (2:1). Cell reorganization and differentiation were characterized by immunohistochemistry and testosterone was quantified by electrochemiluminescence.
Main results and the role of chance: During long-term cultures in 1LSs, testicular cells reorganized into organoids with restoration of testicular architecture and Leydig cell functionality supporting the differentiation of germ cells to the meiotic phase, regardless of the mouse strain. However, pore overgrowth and fusion of adjacent aggregates, resulted in irregularly shaped TOs. Based on these results, the design of 1LS was modified with an additional layer of alginate to entrap reorganizing cells (2LS). To non-invasively evaluate germ cell behavior, EGFP-expressing germline stem cells were mixed with testicular cells of prepubertal C57BL/6J mice in 2LS. This approach resulted in the formation of chimeric organoids with a more regular and spheroidal morphology. These improved TOs consisted typically of 1 tubule-like structure and surrounding interstitium, representing the functional unit of a testis. in contrast to primary germ cells, germline stem cells were not observed after the 3rd week of culture.
Limitations, reasons for caution: Candidate factors have to be tested in their ability to elevate the meiotic blockage of germ cells in TOs. In addition, the culture medium needs further optimization to enhance maintenance of germline stem cells in chimeric models. Finally, results obtained with rodents remain to be confirmed in further human studies.
Wider implications of the findings: The opportunities testicular organoids offer to manipulate cells through genetic modification, inclusion and exclusion, are essential for the study of male infertility and the search for potential therapies. Moreover, they permit high-throughput screening of chemicals, thereby substantially reducing the number of animals for the high demanding reproductive toxicity studies.
Trial registration number: not applicable

P-800 Generation of artificial oocytes by two distinct mechanisms

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Study question: Are haploid genome replication and somatic cell haploidization feasible mechanisms for generating parentally genotyped oocytes?
Summary answer: Artificial oocytes can be generated by haploid genome replication and somatic cell haploidization. The latter is more efficient and capable of generating live offspring.
What is known already: A low number of mature oocytes is one of the major limitations to treating infertile women who have impaired ovarian reserve. Although it has been proposed that competent oocytes can be created by a phenomenon known as somatic cell haploidization (SCH), its clinical value has yet to be examined due to its poorly understood mechanism. On the other hand, spindle transfer has been clinically applied for mitochondrial replacement therapy. Therefore, we propose to utilize G2-phase haploid pseudo-blastomere (HpB), generated by parthenogenesis, as a nuclear donor to create oocyte replica.
Study design, size, duration: In the past 7 months, individual G0 phase cumulus cells (CCs) were transferred into 1,066 ooplasts for SCH. HpBs obtained from the activation of 80 oocytes were transferred into 464 ooplasts. Both cohorts were ICSI-inseminated and placed in the time lapse for embryo development. Another 379 unmanipulated oocytes were ICSI-inseminated, serving as control. Pre-implantation development was monitored and compared for both neogametogenesis techniques. Fully expanded blastocysts were transferred to obtain live pups.
Participants/materials, setting, methods: CCs were isolated from the cumulus oophorus of B6D2F1 mice. HpBs were obtained via oocyte activation, cultured to the 8-cell stage, and subsequently treated by nocodazole to synchronize at the G2-phase. In two experimental groups, CCs or HpBs were individually transferred into the perivitelline space of the ooplasts with inactivated Sendai virus. Reconstructed oocytes presenting with a pseudo-meiotic spindle were fertilized by piezo-actuated ICSI. Blastocysts were transferred into a pseudo-pregnant CD-1 surrogate to obtain pups.
Main results and the role of chance: A total of 1,769 oocytes underwent enucleation to generate ooplasts, with a survival rate of 97%. Survived ooplasts were allocated to SCH (n=1,034) and HpB-SCNT (n=458). To generate HpBs, 80 unmanipulated oocytes were activated; 58 of them progressed to the 8-cell stage and generated 464 HpB for SCNT. For SCH, CCs were selected based on morphology with a diameter <10 micron. Nuclear transfer of CCs and HpB yielded survival rates of 98.6% and 93.2%, respectively. Following SCH and HpB-SCNT, spindle development for SCH and HpB-SCNT was comparable at 63.5% for SCH and 66.7% for HpB-SCNT. The ICSI survival rates for SCH and HpB-SCNT were 58.9% and 64.9%, respectively, but lower than the control at 73.9% ($P<0.001$). Fertilization rates for SCH and HpB-SCNT were also comparable at 61.3% and 64.3%, respectively, but lower than the control at 89.6% ($P<0.00001$). Full pre-implantation development was achieved for both experimental groups. While the SCH group yielded a development rate of 24.6% (n= 94), the HpB-SCNT group yielded a lower rate at 12.4% (n= 23) ($P<0.001$), both lower than the control (71.7%, $P<0.00001$); however, the morphokinetics of the embryo development was retained. To date, only 3 live pups were obtained from SCH group.
Limitations, reasons for caution: While these techniques to manufacture oocytes are very new and highly experimental, our findings show a lower blastulation rate for oocytes generated by HpB. Both techniques require refinement and improvement of reliability and consistency before they can be considered a feasible technique for human reproduction.
Wider implications of the findings: The study confirms the potential to create artificial oocytes capable of supporting full pre-implantation development and, in some cases, live pups. If further streamlining of both procedures demonstrates their safety, they may both represent a viable option to generate *de novo* gametes
Trial registration number: N/A

P-801 Effects of Lonomia obliqua venom components on human endometrial stromal cells: A potential source for new cytoprotective biomolecules against recurrent pregnancy loss

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Study question: Could new molecules like Lonomia obliqua lipocalins and hemolins have cytoprotective effects on endometrial stem cells (hESC)?
Summary answer: Lonomia obliqua venom-induced hESC viability, proliferation and migration occurred mainly by protection against oxidative damage and ERK-dependent pathway activation
What is known already: Recurrent pregnancy loss (RPL) is associated with severe physical and psychological morbidity, for which there is no treatment options. The pathophysiology involves deficiency in proliferation and migration capacities of endometrial stromal cells (hESCs) impairing embryo implantation and development. Animal venoms are rich sources of bioactive molecules and despite its known toxic effects, they also have protective components such as pro-proliferative molecules, growth factor-like, anti-apoptotic and anti-oxidant.
Study design, size, duration: This study was an experimental in vitro with endometrial stem cells. Treatment duration was 8-72h. Every assay had control cells exposed to phosphate buffered saline (PBS).
Participants/materials, setting, methods: hESCs were isolated from fresh human endometrial biopsies and characterized according standard protocols. Then the effects of L. obliqua venomous secretions on cell viability, proliferation and migration were determined using MTT, wound-healing assay, sulforhodamine B (SRB) assay and measuring the immunocontent of Ki67. Venom components involved in cell enhancing effects were also identified by classical chromatographic methods and proteomic analysis. Assays were conducted in triplicate.
Main results and the role of chance: The hESCs in culture showed adhesiveness properties, presented a fusiform fibroblastoid morphology and ability to in vitro differentiate into adipocytes, osteocytes and chondrocytes. The expression of cell surface markers was also characterized by flow cytometry. hESCs were positive for mesenchymal markers (CD105, CD90 and CD73) and negative for hematopoietic markers (CD45 and CD11), indicating that isolated cells have potential for multilineage differentiation. L. obliqua bristle extract (LOBE) increased dose-dependently hESCs viability in a concentration range varying from 0.001 to 0.1 µg/mL, independently of the cell isolation bath. For some cell isolates (patient ID 1, 3, 4, 6 and 7) it was observed a slightly reduction in hESC viability at highest LOBE concentrations (10 µg/mL). Treatment increased hESC viability in the presence of low concentrations of fetal bovine serum (1 % FBS) and even in its complete absence. This effect was long lasting, being significant up to 72 h of incubation with LOBE in serum deprivation conditions. r to identify the potential molecules involved in the cytoprotective action, a mass spectrometry-based proteomic analysis was performed. It was identified a total number of 430 proteins in LOBE and 312 proteins in L. obliqua hemolymph.
Limitations, reasons for caution: This study was only conducted in vitro.
Wider implications of the findings: In this work we reported the identification of at least six protein classes with cytoprotective properties through proteomic analysis and isolated one fraction enriched in this cytoprotective factors. L. obliqua secretions induced increase in hESCs viability, proliferation and migration mainly by the protection against oxidative damage and ERK-dependent pathway activation.
Trial registration number: not applicable

P-802 The fate and regenerative efficiency of differently administered BMSCs in thin-endometrium rat models.

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Study question: This study aims to compare the engraftment, retaining time and therapeutic efficiency of differently administered BMSCs and help to select an optimal therapeutic route in clinical settings.
Summary answer: Compared with intrauterine infusion, BMSCs could better promote angiogenesis by upregulating related cytokines, such as VEGF, when administered through the ipsilateral iliac artery.
What is known already: MSC-based therapy has become a promising method for endometrial disease(thin endomtrium or Ashernmen's syndrowe). Therapeutic effects could always be observed even though different MSC administration routes or MSCs of different tissue sources were used in these studies. Only a few studies compared efficacy of different transplantation routes. However, the results seem to be controversial. Comparable therapeutic effects were reported in some studies, while others stated that systematic administration gave a better outcome than local administration.
Study design, size, duration: Experimental animal study. Forty-eight female Sprague-Dawley (SD) rats were used in this study. They were randomly assigned to 4 groups: normal, injured, intra-arterial and intra-uterine group. For all rats except for normal group, the thin endometrium models were established by infusing 95% ethanol into the uterine horns and BMSCs were transplanted either locally or intra-arterially after modeling. The therapeutic efficacy were evaluated in the following month.
Participants/materials, setting, methods: The thin endometrium models induced by ethanol in SD rats, GFP/Luciferin labeled BMSCs were injected either locally or intra-arterially. The retaining time and quantitative distribution were assessed by in vivo bioluminescence imaging and immune-histological analysis. The precise location and differentiation of differently administered BMSCs were determined by immunofluorescence methods. The endometrial fibrosis, angiogenesis were detected by immunohistochemistry and western blotting at a consecutive time after treatment to compare the therapeutic efficiency of two administration methods.
Main results and the role of chance: The engraftment and differentiation abiility were comparable in 2 groups. The luminescent signal both remained distinct and strong in the abdomen in the first 4 days post-treatment(7.98×105 and 6.02×105p/s for IU and IA group), indicating the precise and concentrated distribution of BMSCs administered both locally or intra-arterially. The luminescent signals disappeared under bioluminescence imaging over time. We further evaluated the precise distribution, differentiation ability and retaining time of the BMSCs delivered in two strategies by immunofluorescence analysis. All the GFP positive cell localized in stroma, but not in the epithelium or myometrium. Furthermore, there are significantly more positive staining in basal layer of the endometrium close to the glands and vessels than the outer layer of the endometrium in the intra-arterial group. At the 28th days post treatment, we could capture a few GFP staining in the basalis layel of endometrium in intra-arterial group and there were no GFP fluorescence signals detected in intra-uterine group($P<0.05$), suggesting a better survival of BMSCs administered intra-arterially. Differentiation ability of differently administered BMSCs were similar. A few BMSCs began to differentiate into stromal cell 12 days after therapy.
Limitations, reasons for caution: No pregnancy tests were carried out in these rats to further confirm the regeneration of thin endometrium and compare the therapeutic efficacy.
Wider implications of the findings: Our study unveiled that the location of MSCs might determined their regenerarive ability and retaining time, and provided an optimal therapeutic route in clinical settings.
Trial registration number: not applicable

P-803 Novel culture conditions for the improvement of the in vitro expansion of human male fetal germ cells

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Study question: Do different ECMs/substrates and growth media culture conditions improve in vitro male human primordial germ cell (hPGC) expansion?

Summary answer: We achieved in vitro expansion improvement of male hPGCs with specific growth factors such as LIF, EGF, FGF2 and GDNF on gelatin- and vitronectin-coating cultures.

What is known already: PGCs are the precursors of male and female gametes, which are specified during early mammalian post-implantation embryonic development. PGCs undergo sequential cell divisions to differentiate into pro-spermatogonia (pSPG). In vitro propagation of pSPG could be important to understand the transition to spermatogonial stem cells (SSCs), important for fertility preservation in patients with infertility. Here, we aimed at performing a comparative analysis on in vitro feeder-free culture systems, based on different extracellular matrix (ECM) and growth media culture conditions, to support the expansion of the male germline stem cell populations using second trimester human male gonads as primary material.

Study design, size, duration: We collected human 2nd trimester male fetal gonads from elective abortions. Male gonads were dissected in saline solution (0.9% NaCl) and were either fixed overnight in 4% paraformaldehyde (PFA) for immunohistochemistry or disaggregated by enzymatic digestion for in vitro culture.

Participants/materials, setting, methods: After differential plating, fetal cells were cultured for 6 days. Disaggregated gonads were cultured with two different growing media (Medium 1 supplemented with LIF, EGF, FGF-2 and GDNF and Medium 2 supplemented with RA, BMP 4 and Activin A) on gelatin, laminin, vitronectin or matrigel coated plates. Cultured cells were immunostained, quantified for the expression of DDX4 and POU5F1 after 3 days (D3) and 6 days (D6) of culture.

Main results and the role of chance: We pursued to evaluate whether germ cells dissociated from a pool of male fetal gonads could propagate in vitro when cultured for D6 in different conditions.

We observed that expansion of POU5F1-positive early PGCs and DDX4-positive late PGCs was only observed when cells were plated on gelatin or vitronectin and cultured with Medium 1, containing the growth factors LIF, EGF, FGF2 and GDNF. However, a reduced percentage of PGCs was observed in all four different coatings when grown with Medium 2, containing RA, BMP4 and Activin A.

We analyzed the relative expression of the PGC markers POU5F1, DDX4 and MAGEA4 in histological sections of gonads from embryos at 18.5 weeks of gestation. Two populations of hPGCs were observed: ~10-30% of the gonadal cells expressed solely DDX4 (late PGCs), whereas less than 10% of gonadal cells expressed POU5F1 (early PGCs). SOX9 and STARD1 expression was evaluated, confirming the presence of Sertoli cells and Leydig cells, respectively.

Limitations, reasons for caution: Due to the limited and difficulty to obtain human fetal tissue, a limited number of samples were used.

Wider implications of the findings: We expanded human male fetal germ cells in vitro for D6 on gelatin and vitronectin coated plates with Medium 1, containing growth factors LIF, EGF, FGF2 and GDNF. Our findings provide a 2D culture system to expand hPGCs that could be useful to study propagation to pSPGs and eventually SSCs.

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P-804 Morphokinetic development by time-lapse imaging of conceptuses generated from artificial oocytes

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Study question: What are the ideal culture conditions to enhance full preimplantation development of embryos generated by FVB somatic cell haploidization (SCH) in the mouse model?

Summary answer: The presence of a histone deacetylase inhibitor yielded the best morphokinetic development of expanded blastocysts generated by FVB SCH, comparable to control blastocysts.

What is known already: Various culture conditions and medium supplements have been proposed to promote preimplantation development of embryos generated by SCH, including supplementation with trichostatin A (TSA), fasudil, scriptaid, and RAD-51 stimulatory compound-1 (RS-1). TSA and scriptaid, both histone-deacetylase inhibitors, have been found to improve embryo development following nuclear transfer by enhancing histone acetylation and cellular reprogramming. Additionally, fasudil is a Rho-associated kinase inhibitor that has been shown to reduce apoptosis and promote cell proliferation. Finally, RS-1 stimulates RAD51 activity, which promotes the repair of DNA damage and increases the efficacy of somatic cell reprogramming.

Study design, size, duration: B6D2F1 mouse metaphase II (MII) oocytes underwent enucleation and nuclear transfer, or were ICSI inseminated serving as controls. Reconstituted oocytes showing development of a meiotic-like spindle demonstrated successful SCH, and were ICSI inseminated. SCH conceptuses were cultured in one of three groups: KSOM, KSOM supplemented with TSA (TSA), or KSOM supplemented with fasudil, scriptaid, and RS-1 (Cocktail). ICSI controls (ICSIC) were cultured in KSOM medium. Fertilization and full preimplantation development were compared among all groups.

Participants/materials, setting, methods: Ooplasts were generated from MII oocytes by removing spindle complexes under OosightÔ visualization and cytochalasin B exposure. A single FVB mouse *cumulus* cell was transferred into the perivitelline space and fused with the ooplast, facilitated by Sendai virus. Reconstructed oocytes with novel pseudo-meiotic spindles underwent piezo-ICSI and were cultured in different media conditions in a time-lapse imaging system up to 96h. TSA and Cocktail embryos had media changed to regular KSOM 10 hours after insemination.

Main results and the role of chance: A total of 274 B6D2F1 MII oocytes were enucleated, resulting in a 95.9% survival rate. All ooplasts survived nuclear transfer and 62.1% successfully haploidized after 2 hours. ICSIC and reconstituted SCH oocytes survived piezo-ICSI at rates of 81.5% and 57.0%, respectively ($P<0.01$). SCH embryos were then allocated into KSOM, TSA supplied, and Cocktail media. Fertilization rates for ICSIC, KSOM, and TSA embryos were 92.4%, 90.7%, and 94.4%, respectively, while the rate for embryos cultured in Cocktail was only 71.9% ($P<0.03$). While embryos cultured in Cocktail had a comparable 2-cell timing to ICSIC, embryos in TSA reached developmental milestones with a closer timing to the ICSIC, having minor delays at the 3-, 4-, and 6-cell stages ($P<0.05$). KSOM- and Cocktail-cultured embryos were delayed at most of the stages ($P<0.01$), except for the two-pronuclei appearance. Although the TSA group displayed the best embryo developmental pattern, the final rate of blastocyst development was somewhat homogeneous with rates of 15.4%, 23.5%, and 13.0% for the KSOM, TSA, and Cocktail groups, respectively ($P<0.001$), and remarkably lower than the ICSIC (81.6%).

Limitations, reasons for caution: Although live pups have been obtained using BDF *cumulus* cells, embryos generated by FVB *cumulus* cells show a remarkably lower blastocyst development, but maintain morphokinetic characteristics similar to ICSIC in the presence of TSA.

Wider implications of the findings: While using different strains to enhance genetic variance, the morphokinetic analysis of preimplantation embryos in ideal culture conditions is paramount to the progress of neogametogenesis. The implementation of this technique may soon help create genotyped oocytes for women with compromised ovarian reserve.

Trial registration number: N/A

P-805 Artificial oocytes: from somatic cells to fertile pups

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Study question: We analyzed the efficacy of generating artificial oocytes using somatic cells (SCs) from two mouse strains (B6D2F1 and FVB) and followed their full pre-/post-implantation development.

Summary answer: While artificial oocytes generated from the new strain (FVB) had higher fertilization rates, those from the standard strain (B6D2F1) provided expanded blastocysts and fertile pups.

What is known already: B6D2F1 is a popular hybrid mouse strain for cloning and transgenic creation due to its geno-/pheno-typic uniformity and high oocyte yield and quality. Indeed, B6D2F1 oocytes have a distinct metaphase II (MII) spindle complex, making them an ideal candidate to generate ooplasts used in SC nuclear transfer (SCNT). However, because they lack genetic variance, they are less suitable for reciprocal SCNT studies. In contrast, FVB mice have single nucleotide polymorphisms and indels on each chromosome that can aid in tracing the pedigree of progeny.

Study design, size, duration: A total of 10 experiments were performed over the course of 3 months, using 30 stimulated mice. SCs were retrieved from cumulus oophorus harvested from FVB and B6D2F1 mice. SCs from both strains

were injected into enucleated MII B6D2F1 oocytes. Unmanipulated B6D2F1 oocytes were piezo-ICSI inseminated, serving as controls. The occurrence of haploidization, fertilization, and full preimplantation development was compared. Some blastocysts were transferred into pseudo-pregnant CD-1 mice to obtain offspring.

Participants/materials, setting, methods: Oocyte enucleation was performed under Oosight™ visualization and cytochalasin B exposure. An FVB or B6D2F1 SC was transferred into the perivitelline space of the ooplast with Sendai virus to promote fusion. Haploidization was monitored by pseudo-meiotic spindle formation followed by extrusion of a pseudo-polar body after insemination. Conceptuses were cultured in a time-lapse imaging system, with piezo-ICSI controls. Expanded blastocysts were transferred into uterine horns of pseudo-pregnant mice. Offspring were mated to test their fertility.

Main results and the role of chance: FVB (n=278) and B6D2F1 (n=905) SCs at G0 phase, with a diameter <10 mm, were chosen for SCNT and transferred into enucleated B6D2F1 ooplasts. Enucleation of 1,212 oocytes yielded a survival rate of 97.6%. Both FVB and B6D2F1 SCNT resulted in similar survival rates of 100% and 98.5%, respectively. Successful haploidization, determined by the presence of a pseudo-meiotic spindle 2 hours after SCNT, was also comparable, with 59.9% of FVB and 63.7% of B6D2F1. Survival after piezo-ICSI was also comparable between FVB- and B6D2F1-reconstituted oocytes, with rates of 64.3% and 60.3%, respectively, albeit lower than the control (75.2%, $P<0.00001$). FVB embryos fertilized at a rate of 88.7%, comparable to the control zygotes at 85.8%, while B6D2F1 conceptuses demonstrated a lower fertilization rate (70.8%, $P<0.00001$). Blastulation of FVB- and B6D2F1-derived embryos was 15.1% and 24.0%, respectively, while the control was 80.7% ($P<0.00001$). Whole-genome karyotyping of 9 B6D2F1-derived blastocysts confirmed 5 of the samples to be euploid. FVB blastocysts (N=8) and B6D2F1 blastocysts (N=81) were transferred into pseudo-pregnant mice, resulting in 3 fertile offspring only from the B6D2F1 conceptuses.

Limitations, reasons for caution: This is still a limited number of observations, and pups were delivered only from the B6D2F1 strain. The utilization of a strain with higher genetic variance may help facilitate offspring fingerprinting.

Wider implications of the findings: This study demonstrates the ability to generate artificial genotyped conceptuses, yielding live offspring. The identification of a feasible donor cell, together with optimization of cell cycle stage and standardization of post-implantation development, will help promote this technique for human reproduction in couples with age-related infertility or poor ovarian reserve.

Trial registration number: N/A

P-806 Neogametogenesis via oocyte replication

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Study question: Can full preimplantation embryo development be achieved from artificial oocytes created through nuclear transfer of a haploid pseudo-blastomere (HpB) into a recipient ooplast?

Summary answer: It is feasible to replicate the female genome and generate novel sibling oocytes that can yield full preimplantation embryo development, albeit at a reduced rate.

What is known already: A limitation of assisted reproduction is the number of available oocytes for embryo creation. It is feasible to utilize a somatic cell nucleus to construct novel oocytes through a process known as haploidization, in which a reverse meiosis occurs after SCNT. Similarly, producing haploid parthenogenetic constructs can generate HpBs, useful for genetic testing at the pre-fertilization level or for reproduction. It is feasible to use a HpB as a nuclear donor since it has already completed homologue segregation.

Study design, size, duration: This is prospective translational animal model study. Over 6 months, 556 oocytes were manipulated for the experimental group, and 158 control oocytes were employed. B6D2F1 HpBs were used to establish the procedure and acquire expertise. FVB HpBs were subsequently introduced for genetic variance. Experimental and control embryos were cultured in a time-lapse incubator (up to 96h). Cleavage parameters were compared to control. Two-sample T-tests and one-way ANOVA with Bonferroni correction were employed for statistical analysis.

Participants/materials, setting, methods: A cohort of oocytes was harvested from B6D2F1 or FVB superovulated mice and artificially activated by 8% ethanol. At the 8-cell stage, HpBs were exposed to nocodazole. Another cohort of B6D2F1 oocytes was enucleated for recipient ooplasts. HpBs were individually transferred into the perivitelline space of the ooplasts alongside inactivated Sendai virus. After fusion, reconstructed oocytes with spindle development were fertilized by piezo-actuated ICSI using B6D2F1 spermatozoa. Unmanipulated and fertilized B6D2F1 oocytes served as control.

Main results and the role of chance: A total of 158 control oocytes underwent ICSI with a 67.7% survival rate; of these, 65.4% developed to the blastocyst stage. For artificial oocyte activation (AOA), up to 10 oocytes were activated for each experiment, yielding 8 HpBs per activated oocyte. For the experimental group, 556 oocytes underwent enucleation with a 96.4% survival rate. Nuclear transfer of HpBs resulted in a 93.2% survival rate, consistent for those derived from BDF and FVB. Reconstructed oocytes showed appropriate development of a novel pseudo-meoitic spindle at a rate of 63.7% for B6D2F1 HpBs and 75.5% for FVB HpBs, and ICSI yielded a 67.1% and 57.7% survival rate, respectively. The fertilization rate for the reconstructed oocytes was 64%. Control oocytes underwent ICSI with a 67.7% survival rate. When evaluating time-lapse parameters, reconstructed embryos created via blastomere nuclear transfer showed asynchrony compared to controls beginning as early as the stage of pronuclear fading. While the majority of reconstructed embryos arrested at the 4-cell stage, of those that progressed, 11.3% of those using BDF HpBs and 14.6% of those using FVB HpBs developed to the fully expanded blastocyst stage. This corresponds to a total of 23 reconstructed embryos that developed to the morula or blastocyst stage.

Limitations, reasons for caution: While we used single-well embryoscope culture for morphokinetic data collection, group culture is superior to single-embryo culture for mice. Thus, developmental rates may be underestimated by this protocol. Implantation and successful pregnancy are also needed to support the clinical utility of this method in generating gametes.

Wider implications of the findings: For women with diminished ovarian reserve, oocyte yield and age-related aneuploidy are limitations to achieving genotyped offspring. Nuclear transfer of HpB can generate sibling oocytes while maintaining genetic information. This model represents a promising path for expanding oocyte yield, allowing genetic assessment of sibling oocytes, and enhancing chances of procreation.

Trial registration number: none

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